CEREAL CHEMISTRY

Published bi-monthly by

The American Association of Cereal Chemists

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Entered as second class matter at the post office at Minneapolis, Minn., under the Act of August 24, 1912. Acceptance for mailing at special rate of postage provided for in paragraph (d-2), Section 34.40, P. L. & R. of 1948, authorized February 10, 1934. Published at 500 So. 5th St., Minneapolis, Minn.

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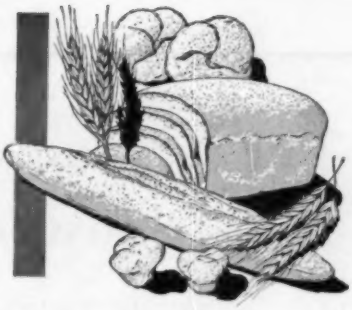

A Brief History of Milling and Baking by SCIENCE WRITER

CHAPTER IV. *The Business of Milling Begins.*

MILLING LEAVES THE HOME

Beginning about 1000 B.C. in Eastern Mediterranean countries an improvement in grinding methods caused an increase in output so that a man's labor produced more grain than his family needed. He had a small surplus to sell or exchange. Thus, milling began to move from the home and into a commercial activity.

THE GREEKS FOUND A WAY

Then about 500 B.C. the Greeks, who were not burdened by the orthodox attitude of the Egyptians and Mesopotamians, experimented with new grinding methods and tools. Within less than 100 years these alert people found much more efficient ways to mill.

In fact, the new devices were so productive that they were essentially industrial machines, housed in factories. These Grecian enterprises were really a combination of miller and baker. Grain was milled to flour, then bread was baked from that flour. Men (mostly slaves) and animals powered the mills.

ROMAN BREADS AND MILLER-BAKERS

The Romans, who were slow to adopt new ideas, finally took to themselves the practice of public milling from the Greeks and then developed it to such a degree that one bakery in Rome produced between 100,000 and 150,000 loaves daily. Even by today's standards that was production on a large scale.

The Roman breads were forerunners of ours. Their loaves were round, about 2 inches high, and were made from kneaded doughs in which leavens and carefully sifted flour of the bread wheats were used. However, it would be difficult to market them today in competition with our featherlight, enriched white bread; they were so heavy that the loaves sank in water.

THE HEALTH OF MILLIONS

Since the days of Greece and Rome when the foundations were laid for today's great baking and milling industries, the world has gained much knowledge. It is remarkable that in all that time the science of nutrition has flourished only within the last fifty years or so. An accomplishment of this science, and the millers and bakers who adopted it, is the enrichment of white flour and white bread with vitamins B_1 and B_2, niacin and iron. Thus is the health of countless millions of people young and old, throughout the world benefited.

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The next chapter titled: "The Evolution of Millstones" will be published soon.

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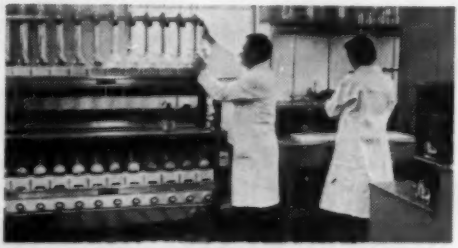

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CEREAL CHEMISTRY

VOL. XXXII JANUARY, 1955 NO. 1

GRAIN STORAGE STUDIES. XVI. INFLUENCE OF STORAGE CONDITIONS UPON THE FUNGUS FLORA OF BARLEY SEED[1]

JOHN F. TUITE[2] AND CLYDE M. CHRISTENSEN[3]

ABSTRACT

Fungi such as Alternaria, Cladosporium, and Fusarium were common and sometimes abundant in barley seed before the seeds matured, but no significant numbers or amounts of Aspergillus or Penicillium were found in the seeds prior to harvest. In seed stored at moisture contents of 10–13% (wet weight), Aspergillus and Penicillium remained static. At seed moisture contents of 13.8–14.2%, *Aspergillus restrictus,* a slow-growing member of the *A. glaucus* group, gradually invaded the germs. At moisture contents of 15–17%, subspecies of the *A. glaucus* group such as *A. repens, A. amstelodami,* and *A. ruber* became the dominant flora, and often the only fungi present. Tests with mold-free and mold-inoculated barley stored at moisture contents favorable to the growth of Aspergillus spp. indicate that invasion of the seed by these fungi can cause or greatly contribute to decrease in seed germination.

Extensive work with wheat, corn, cottonseed, and rice (2, 4, 5, 7, 8, 9, 15, 16, 17) has shown that species of Aspergillus and Penicillium, capable of growing at moisture contents in equilibrium with relative humidities of 65 to 90%, are associated with, and often primarily responsible for, a variety of deteriorative changes in moist stored grain. So far as the writers are aware, no work of this kind has been done with seed of malting barley. During the past few years occasional commercial parcels of barley were encountered which had been stored in the fall as malting grade, and which had deteriorated to feed grade by late winter or early spring. *Aspergillus glaucus* and *A. candidus,* both common storage molds, were isolated in large numbers from some of these samples. The relation of these and related fungi to the deterioration of stored malting barley seemed, therefore, to warrant some study, and so the work here reported was undertaken.

[1] Manuscript received September 8, 1954. Paper No. 3208, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota. A portion of the work here presented was included in a thesis submitted by the senior author to the Graduate School, University of Minnesota, in partial fulfillment of the requirements for the degree of Master of Science. The work on which this report is based was supported in part by a grant from the Kurth Malting Co., Milwaukee, Wisconsin.

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Materials and Methods

Source of Barley Seed. The seed came principally from 1) parcels stored in commercial bins, originating mostly on farms in Minnesota and North Dakota; 2) malting varieties grown in experimental plots of the University of Minnesota; and 3) varieties Kindred and Moore grown in irrigated plots at Aberdeen, Idaho.[4] This seed was nearly free of Aspergillus and Penicillium, and contained very few other types of fungi.

Germination. One hundred to 400 seeds were placed on moist germination blotters and kept at room temperature; germinated seeds were counted after 5 and 7 days.

Moisture Contents. Either the one-stage or the two-stage air-oven method, as specified in Cereal Laboratory Methods (1), was used. The one-stage method was used only when the moisture content of the sample was known to be below 13.0%.

Numbers and Kinds of Fungi on and Within the Seed. The chief methods used were:

1. The mold count procedure described by Bottomley *et al.* (2). In this, 5 g. of seed are suspended in 500 ml. of 0.2% sterile agar solution in water and comminuted in a Waring Blendor for 1 minute; the suspension is diluted to various concentrations in 0.2% sterile agar solution, and aliquots are cultured in various agar media. This determines chiefly the number of viable spores present; counts obtained by this method are referred to as "spore count" or "mold count."

2. Seeds are washed in running water, or submerged for 1 minute in 1% sodium hydrochlorite solution, then rinsed in sterile water and cultured. This reveals the number of seeds that contain living fungi, but gives no information on the amount of fungus present or its location within the seed.

3. Seeds are surface-disinfected with sodium hypochlorite solution as in 2 above, the lemma and palea are removed aseptically, and they as well as the caryopsis are cultured separately. This gives some idea of the kinds of fungi present in various portions of the seed.

4. Seeds or portions of them are sectioned, either free-hand or with a microtome, using the method described by Swaebly (19); and are stained and examined microscopically. This gives some information on the location and amount of mycelium present, but little or no indication of its identity.

Culture Media. As the composition of the medium may influence

[4] Obtained through the courtesy of Drs. Harlan Stevens and G. A. Wiebe, U. S. Department of Agriculture, Beltsville, Maryland.

the kinds of fungi isolated from seed (2, 13), several media, principally Czapek's, acid potato-dextrose, and malt-salt agars were used. Of these, the malt-salt agar was used most extensively, because preliminary tests, in which more than 20 different seed lots were cultured on the three media listed, showed that appreciably greater numbers of storage molds (Aspergillus and Penicillium) were obtained on this than on the other two. This agrees substantially with results reported by Bottomley *et al.* (2).

Storage of Test Samples at Different Moisture Contents. In most cases, 100 to 300 g. of seed were stored in 1-qt. Mason jars equipped with screw caps and rubber rings. The moisture content of the test seed was determined, and the amount of water required to bring the seed to the desired moisture content was added. The jars were closed, stored at 5°C. for several days, and shaken occasionally during this time to ensure even distribution of moisture. The moisture content was determined at that time, and usually when test portions were subsequently removed from the jars.

Results

Fungus Flora of Barley Seed Prior to Harvest. Hyde (10) found mycelium to be present commonly, and often in abundance, beneath the pericarp of wheats from the major wheat-growing areas of the world. Hyde and Galleymore (11) showed that mycelium usually is present in developing wheat seed from the soft dough stage onwards. They isolated principally Alternaria from strips of pericarp from mature seed. Christensen (3) cultured pericarp strips from commercial samples of wheat and found most of the mycelium on the inner side of the pericarp to be dead. Most of the living mycelium in the high-grade lots was that of Alternaria, while in the low-grade lots it was mostly Aspergillus and Penicillium. He did not determine when Aspergillus and Penicillium had entered the seed. It is, of course, of some importance to know whether most of the invasion of the seed by typical storage molds such as Aspergillus and Penicillium occurs before or after harvest.

Mature seeds of five varieties were collected just before harvest in 1951 at four branch stations of the University of Minnesota. The seeds were washed in running water, rinsed in sterile distilled water (preliminary tests showed that this removed most of the external contaminants), blendorized, and diluted as described above under the heading "Mold count" in the previous section, and cultured on malt-salt and acid potato-dextose agars.

No storage molds were found in any of the 15 samples so cultured.

All of the cultures were in duplicate plates at dilutions of 1:1000. If viable spores of Aspergillus and Penicillium had been present in amounts of more than a few hundred per g. of seed, it is likely that they would have been detected. From 14,000 to 225,000 colonies per g. of seed were obtained of the fungi Alternaria, Cladosporium, and Candida sp. None of these are known to grow in stored seeds whose moisture content is below 20%. Counts of yeasts other than Candida sp. ranged from 3500 to 420,000 per g. of seed.

During the growing season of 1952, seeds of the variety Kindred were gathered from test plots of the University of Minnesota's Institute of Agriculture at St. Paul every second day, from the boot stage until after the seeds were ripe, and from lodged and upright plants left for 2 weeks after normal harvest time. One hundred seeds collected at each sampling period were washed in running tap water, rinsed in sterile water, and cultured on malt-salt agar. Another 100 seeds of each were surface-disinfected in 1% sodium hypochlorite solution for 1 minute, rinsed in sterile water, and cultured on malt-salt agar. These procedures should have served to detect even small amounts of living mycelium on or within the seed.

From the 1200 seeds collected at six different periods, almost no fungi other than Alternaria, Cladosporium, and Fusarium were obtained. *Aspergillus flavus* grew from some of the ripe seeds collected on August 9. This apparently was due to chance contamination during the culturing process, since only 4% of the washed seeds yielded *A. flavus*, while this fungus grew from 14% of the surface-disinfected seeds. No colonies of any other species of Aspergillus grew from any of the seeds, and Penicillium grew from only a few seeds collected at one period.

The 1952 harvest season in the locality where these plants were grown was unusually moist, with frequent showers; relative humidities above 75% often prevailed for several days at a time. The grain sampled 2 weeks after maturity was partly lodged, and thus was exposed to conditions that presumably would favor infection of the seeds by fungi.

The results provide fairly convincing evidence that the seeds were not invaded to any appreciable extent by Aspergillus or Penicillium prior to harvest. Similar evidence, as yet unpublished, with wheat during the past several seasons supports the contention that these seeds are not invaded to any great extent by these same genera while the seeds are still in the heads, regardless of the weather conditions during ripening.

Where and in What Form Fungi Occur within Barley Seed. Freshly

harvested, bright seeds of several varieties of malting barley were washed in running water, then various portions of the seeds were removed, stained with aniline blue, and examined microscopically. Large amounts of mycelium (Fig. 1) and clusters of what appeared

Fig. 1. Mycelium on the inner side of the lemma of a barley grain.

to be yeast cells were present on the inner side of the lemma and palea. Mycelium frequently was present, but less abundant, in and within the pericarp. Mycelium was moderately abundant within the husks of even the seed from plants grown under irrigation in Idaho, but apparently none of this was mycelium of storage molds. Some seeds from various sources were embedded, sectioned, and stained. Abundant mycelium was observed in the parenchyma cells of the inner part of the husk of many of these, as shown in Fig. 2. In every seed lot so far examined, mycelium has been at least moderately abundant on the inner side of the lemma and palea. The results given in the previous section strongly indicate that the mycelium present up to the time

Fig. 2. Longitudinal section of grain coats of barley showing mycelium in parenchyma cells of the husk.

of harvest is not that of Aspergillus and Penicillium. Thus, the mere presence of abundant mycelium within the seed is not evidence that molds significant in storage deterioration have invaded the seed.

Relation of Moisture Content of Barley Seed to Invasion of the Seed by Fungi Significant in Storage Deterioration. Moisture content is known to be of major importance in determining the kinds of fungi that invade stored seed and the degree to which they invade it (12, 16). Data by Coleman and Fellows (6) indicate that the critical moisture content for safe storage of barley is in the range of 14.0–14.5%. However, the fact that at least one species of Aspergillus commonly found in seed can grow in materials whose moisture content is in equilibrium with a relative humidity of about 67% (18) would lead one to suspect that a moisture content of 14.0–14.5% might be somewhat high for safe storage of barley over long periods.

Commercial samples of malting barleys were stored as described in the section "Materials and methods," at moisture contents of 10.0,

12.0, 12.5, 13.2, 13.8, 14.0, 15.0, and 16%, for up to 18 months. Samples were removed periodically and tested for moisture content, number and kinds of molds present, and germination. In all cases duplicate moisture contents agreed within 0.2%.

At moisture contents of 10.0 to 13.2% there was no detectable change in either the spore count or the number of surface-disinfected seeds yielding various species of Aspergillus and Penicillium.

At moisture contents of 13.8–14.2% there was a slow, gradual increase in percentage of seeds yielding a slow-growing subspecies of the *A. glaucus* group tentatively placed in the *Aspergillus restrictus* series (20). This fungus frequently was restricted to the germ. It grew very slowly on potato-dextrose and on the ordinary malt-salt agar. When seed that had been stored for a year at moisture contents of 13.8–14.2% was cultured on an agar medium containing 2% malt extract and 20% sodium chloride solution, this fungus grew slowly from, and fruited sparsely on, the germs. Often it could be detected only by examination of the seed with a stereoscopic microscope, × 10, after the cultured seed had been incubated for 10 to 14 days. This fungus had not sporulated on most of the seeds that it had invaded, prior to the time the seed was cultured. Its presence could not be evaluated by spore counts or by other methods that depend upon obvious sporulation. The same fungus has since been encountered commonly in wheat stored both in the laboratory and in commercial bins at moisture contents slightly below 14%. Its significance in the deterioration of grain stored at and just below the moisture contents considered critical for safe storage is now under investigation.

At moisture contents of 15–16%, various subspecies of the *Aspergillus glaucus* group, especially *A. repens, A. amstelodami,* and *A. ruber,* increased rapidly and eventually became not only the predominant, but almost the only, organisms present or detectable by the technics used. At these moisture contents, the "field" fungi such as Alternaria, Cladosporium, and Fusarium originally present in some of the seeds disappeared.

Effect of Storage Molds on Viability of Barley Seed. There is considerable evidence that at moisture contents up to 18%, invasion of cereal seeds by storage molds is associated with decrease in germination of the seed (2, 5, 8, 17). Hummel *et al.* (9), in studies of nearly mold-free and mold-inoculated wheat, concluded that storage molds were responsible for the major deteriorative changes encountered in moist stored wheat. Christensen and Drescher (4) found that increase of storage molds accompanied or preceded decrease in germination of the seed. It is known (14) that storage of seed under high concen-

trations of carbon dioxide or a temperature of 35°C., with high moisture contents, even in the absence of molds (9) will result in death of the seed. The fact that processes or conditions other than those initiated or stimulated by molds can weaken or kill seeds does not lessen the significance of the findings that when storage molds invade the germs of seed they may cause or largely contribute to weakening and death of the seed. To explore this, several tests were made with various barleys.

Seeds of the variety Kindred, grown under irrigation at Aberdeen, Idaho, were washed in running tap water (not surface-disinfected) and cultured on malt-salt agar. They yielded a few colonies of Alternaria and Cladosporium, but none of Aspergillus or Penicillium. Samples of this seed were then surface-disinfected with 1% sodium hypochlorite solution for 1 minute, and dried to 13% moisture content by means of sterile dry air passed through them. Portions of 150 g. were transferred to sterile 1-qt. jars. To one series, sterile water was added to give the desired moisture contents. To another series a suspension of spores of *Aspergillus glaucus, A. candidus, A. flavus,* and Penicillium was added, to give a total of approximately 30,000 spores per g. of seed, and the moisture contents were adjusted to 16–19.4%. The seeds were stored for 15 to 30 days at room temperature, and the viability of the seed, and percentage of seeds infected with various molds, were determined. The results are summarized in Table I.

TABLE I

EFFECT OF STORAGE MOLDS ON THE VIABILITY OF KINDRED SEED STORED AT ROOM TEMPERATURE

Treatment	Moisture Content	Storage Time	Seed Viability	Seed Infected with:		
				A. glaucus	A. candidus	Penicillium
	%	*days*	%	%	%	%
No molds[a]	13.1	30	99	1	0	1
Molds[b]	13.0	30	97	1	0	0
No molds	16.6	30	96	0	0	0
Molds	16.7	30	76	73	11	6
No molds	18.4	30	94	0	0	0
Molds	17.4	30	81	85	15	6
No molds	19.0	15	100	0	0	0
Molds	19.0	15	73	41	11	21
No molds	19.4	15	98	0	0	0
Molds	19.4	15	72	29	15	21

[a] Seeds were surface-disinfected with 1% sodium hypochlorite solution for 1 minute and aseptically air dried; calculated amounts of sterile water were added to bring them to desired moisture content.

[b] Seeds were inoculated with a 5-ml. water suspension of storage molds before storage.

No loss of germination occurred in the seeds stored at these high moisture contents in the absence of storage molds. The germination of the samples inoculated with storage molds decreased 18–27%. At the end of the test, *Aspergillus glaucus* was the principal fungus present in those samples inoculated with a mixture of mold spores, but some *A. candidus* and Penicillium were present in all of the inoculated samples. This furnishes experimental evidence that invasion of barley seed by storage molds will result in reduction in germination of the seed.

A number of tests was made with various commercial lots, stored at various moisture contents between 13 and 18%, and for various lengths of time. Most of these gave essentially the same results. Results of a typical test are summarized in Table II.

TABLE II

THE NUMBERS AND KINDS OF STORAGE FUNGI AND GERMINATION OF A COMMERCIAL BARLEY SAMPLE STORED AT MOISTURE CONTENT OF 13.7 TO 16.6% AT ROOM TEMPERATURE FOR 7 MONTHS

Moisture Content	Seed Infected by		Viability
	A. glaucus	Penicillium	
%	%	%	%
13.7	66	9	91
14.0	48	1	88
15.3	80	13	55
15.7	86	15	52
16.6	85	23	7

At a moisture content of 13.7%, *Aspergillus glaucus* remained about constant for 7 months, and at a moisture content of 14.0% *A. glaucus* decreased. At the time these tests were made, however, the *A. restrictus* mentioned above was not known to us, and it is probable that this fungus may have been increasing in both of these lots. At moisture contents of 15 to approximately 17%, invasion by *A. glaucus* and Penicillium increased, and germination of the seed decreased greatly. These results are in general agreement with those reported for the mold-inoculated samples in Table I and support the contention that invasion of barley seed by storage molds causes or contributes to decrease in germination.

Discussion

Immature seed of barley may be invaded by a variety of fungi during the growing season, but none was invaded appreciably prior

to harvest by such common storage molds as Aspergillus and Penicillium spp. Thus when parcels or lots of barley seed are encountered that are heavily invaded by species of Aspergillus or Penicillium, the principal genera of storage molds, it seems safe to suppose that this invasion occurred mainly after harvest.

In seeds stored for as long as 18 months in the laboratory, no invasion of the seed by microorganisms could be detected at moisture contents of 13.2% and below. At moisture contents just above and below 14%, seeds stored for some months were invaded by *Aspergillus restrictus*, a slow-growing member of the *A. glaucus* group. This fungus was very difficult to detect by any of the culture technics used, and almost impossible to detect by any other means. Its role in the deterioration of seed is not known, but seems to warrant further study. At moisture contents of 15 to 19%, *A. repens*, *A. amstelodami*, and *A. ruber*, all subspecies of *A. glaucus*, and all known to grow on and in other kinds of cereal seeds at similar moisture contents, often became the predominant, and sometimes the only fungi present. All of these species of the *A. glaucus* group, as well as *A. candidus* and Penicillium, invade various parts of the seed, including the germ, and directly cause or greatly contribute to reduction in germination.

So far as the writers know, *A. restrictus* is reported here for the first time as an invader of stored seeds. The difficulty with which this fungus was detected in, and cultured from, the seeds suggests that determination of number and kinds of fungus flora in any given lot of seeds may require extensive and thorough testing.

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DEVELOPMENTAL MORPHOLOGY OF THE KERNEL IN GRAIN SORGHUM[1]

E. H. SANDERS

ABSTRACT

Growth of the endosperm, seed coat, and pericarp of the grain sorghum kernel was traced from the time of pollination until maturity. Histological sections revealed that, in the endosperm, an epidermis and a subepidermal layer distinct from other cells were formed a few days after pollination. Starch deposition in the crown endosperm and production of oil in the epidermis ocurred shortly after this initial growth. Varietal differences were not observed in endosperm development except for the production of waxy starch in one variety.

A layer of cutin between the integuments at the time of pollination, as well as the inner integument between the endosperm and pericarp, were transformed into a seed coat. In most varieties the cutin layer became thicker and cellular layers disappeared as the kernel matured. In all varieties, the origin of the seed coat was traced to the integument and not to the nucellus as has been reported previously.

Growth in the pericarp was confined to cell enlargement. Starch deposition in the mesocarp continued as the kernel matured and prevented complete collapse of this region as in other cereals such as corn.

The development of structures in the grain sorghum kernel is important to millers as well as to grain growers. Improvements in technics of milling and objectives in grain sorghum breeding are dependent on a knowledge of structure in the kernel. In areas where corn will not grow well, crop substitutes such as grain sorghum are fulfilling the needs of grain millers. Both dry and wet millers need information similar to that available on corn (10).

Because milling technics are concerned with the separation of various kernel structures, a study of developmental morphology is of particular interest. Microscopic examination of the mature kernel will not reveal structures as easily as following the development of structures within the kernel. For example, the seed coat was identified as the remains of the nucellus (8) in 1928, based on earlier work (9) which described the anatomy of mature grain sorghum. More recently (in 1949) this structure was called the testa and its origin was correctly described (1). However, varietal differences were not studied. These varietal differences have proved to be of great importance to the grain processor. The pigmented seed coat, called the undercoat in the wet milling industry, and the problem it has presented in some varieties have been discussed in the literature (11).

[1] Manuscript received May 27, 1954. Contribution from the George M. Moffett Research Laboratories, Corn Products Refining Company, Argo, Illinois.

The reproductive structures of the grain sorghum kernel, prior to pollination, and subsequent growth of the germ have been described (1). Conner and Karper (2) and Swanson (8) studied inheritance of seed coat color. Winton (9) described the mature kernel of several species of sorghum. However, no investigation has given an account of the development of the grain sorghum kernel from a morphological standpoint.

The purpose of the present work is to trace the development of the endosperm, seed coat, and pericarp to gain a more thorough understanding of the kernel structures important in wet milling. The morphological terms used are the same as those used for corn by Wolf *et al.* (10).

Materials and Methods

The studies were conducted on six grain sorghum varieties grown at Argo, Illinois, from seed furnished by the Texas Branch Agricultural Experiment Sub-station No. 8 at Lubbock, Texas. Martin, Early Hegari, and Combine Kafir 60 were grown in the summer of 1951; Texas Combine Kafir, Combine Kafir 54T, and Texioca 54 were grown in the summer of 1953. Material was tagged a few hours after pollination and collections were made at 3-day intervals. Kernels representing different stages of development were preserved in Craf solutions (7). Freezing microtome sections 40μ thick were used for preliminary examinations and for microchemical tests. Sudan IV and iodine-potassium iodide solution were used in tracing morphological development of immature kernels.

Sections 10 to 14μ thick were prepared by embedding in paraffin, staining in safranin, and counterstaining with Fast Green or Orange G. Nuclei were stained red with safranin while cytoplasm was stained with the counterstains.

Anatomy of the Mature Kernel

The mature seed of grain sorghum consists of the embryo, or germ, and the endosperm, both surrounded by a seed coat. This seed is enclosed in the pericarp, or outer covering. The whole structure is called a caryopsis, or kernel, and is borne singly on a pedicel, or stalk. Although they differ in manner of attachment to the vegetative plant, grain sorghum and corn kernels have the same structural parts. This investigation is concerned with post-fertilization development of these kernel parts, except for the embryo. Artschwager has given an excellent description of the latter (1). The parts of the kernel are given in Fig. 1.

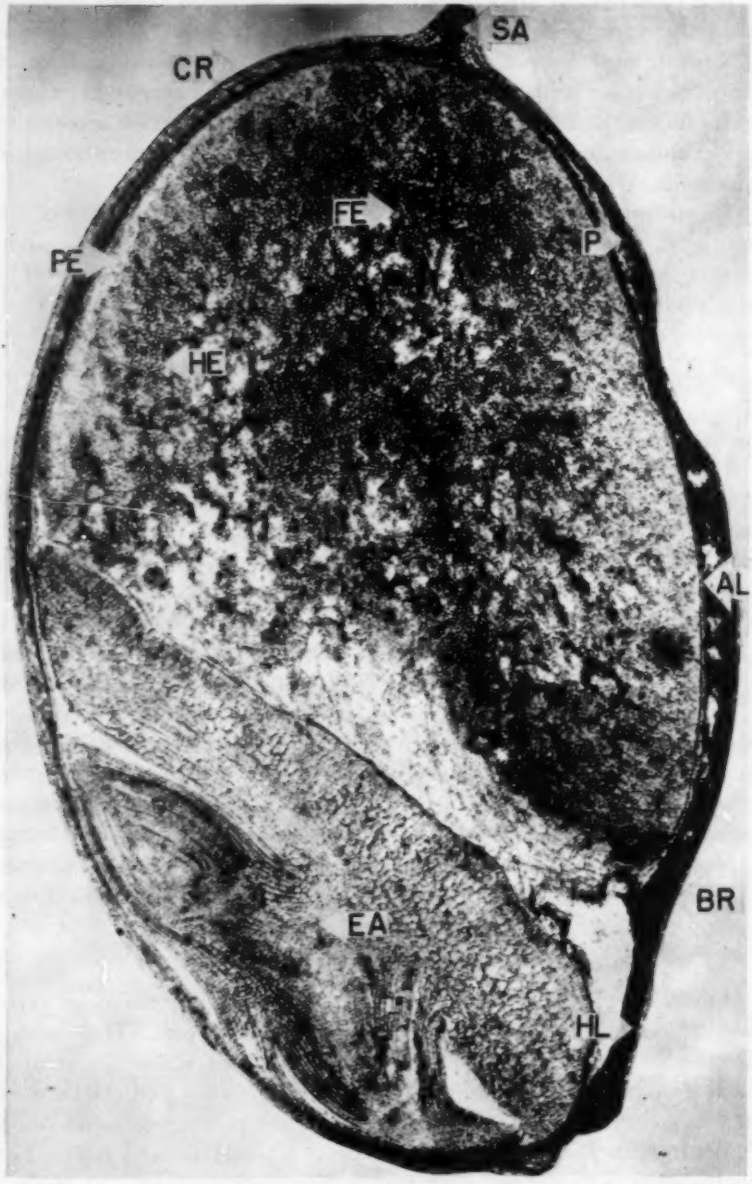


Fig. 1. Median longisection perpendicular to face of kernel at maturity. SA, silk attachment; P, pericarp; AL, aleurone; PE, peripheral endosperm; FE, floury endosperm; HE, horny endosperm; HL, hilar layer; EA, embryo axis; CR, crown region; BR, basal region. 20 ×.

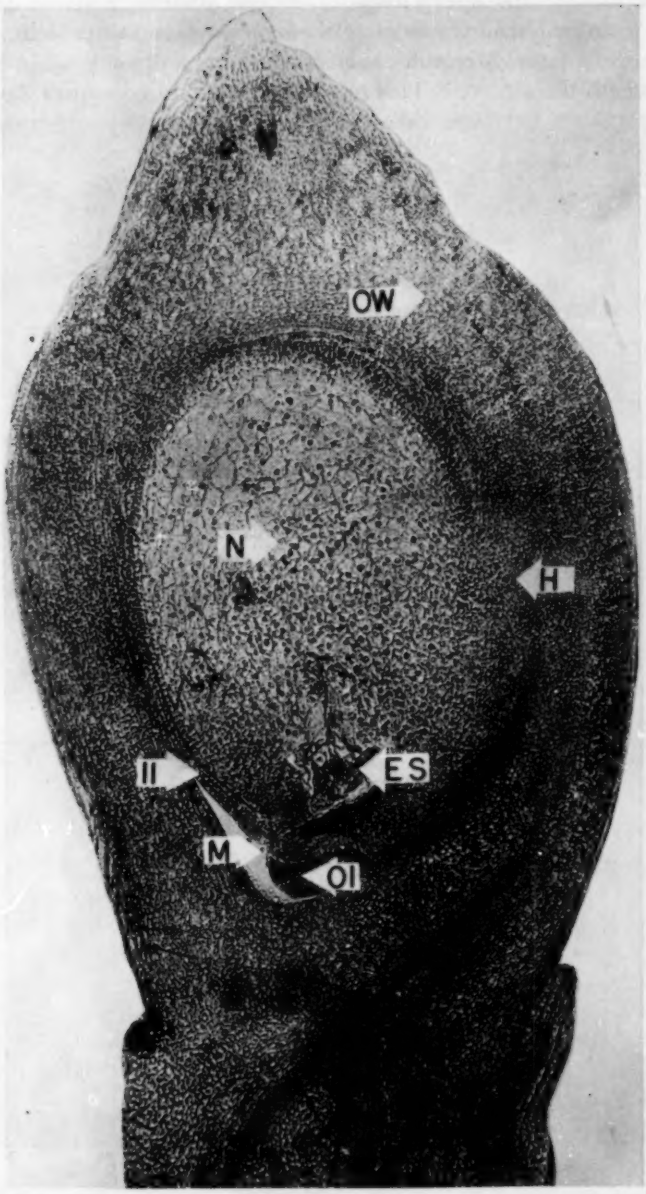


Fig. 2. Longisection at time of pollination. OW, ovary wall; OI, outer integument; II, inner integument; N, nucellus; ES, embryo sac; M, micropyle; H, hilum. 31.5 ×.

The mature endosperm consists of cells filled with starch and includes a single outside layer of cells containing oil and protein, called the aleurone layer. A region of cells containing a dense protein matrix lies beneath the aleurone. This region of two to six concentric layers in grain sorghum has been called the peripheral endosperm layer, and

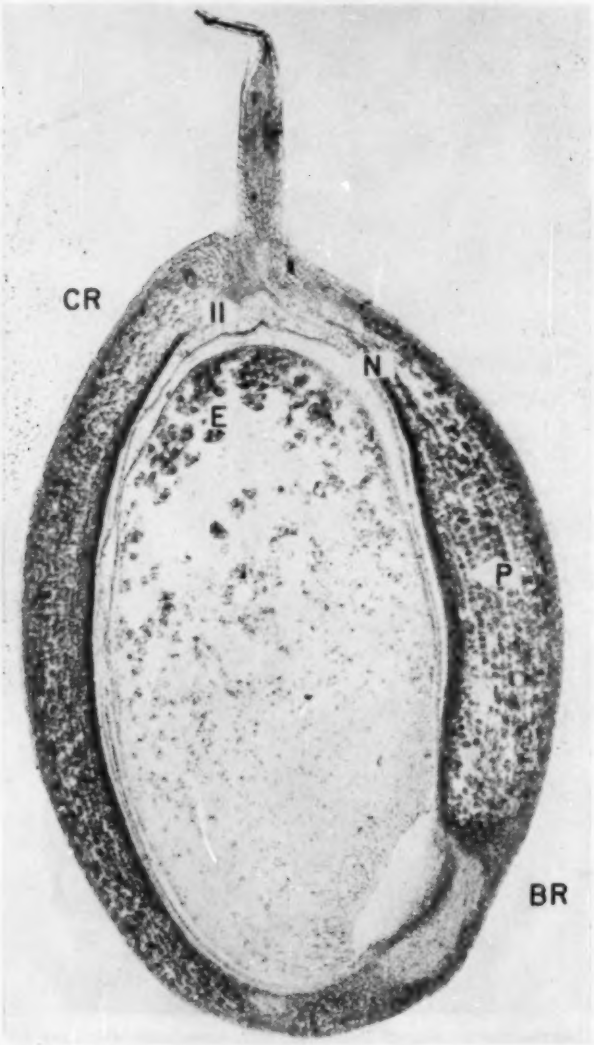
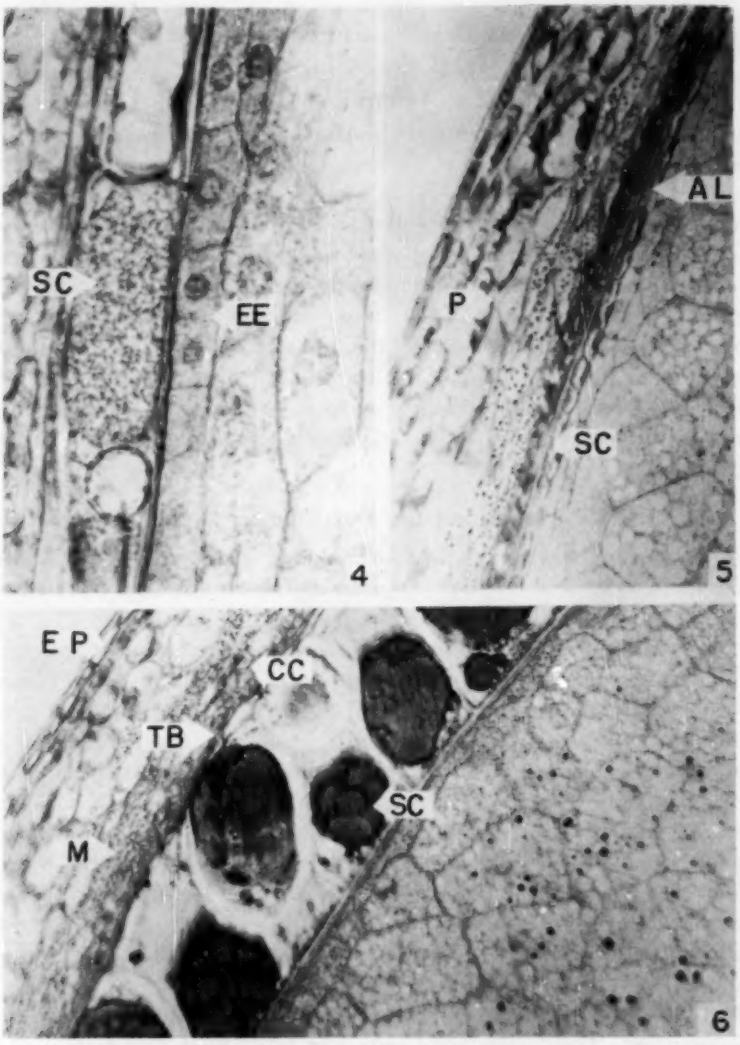


Fig. 3. Longisection 9 days after pollination. P, pericarp; II, inner integument; N, nucellus; E, endosperm; CR, crown region; BR, basal region. 25 ×.

its relation to wet milling will be discussed in detail in another publication. The endosperm cells which store starch are further divided into an outer horny region and an inner floury region.



Figs. 4, 5, and 6. Fig. 4 (top, left): Transection of seed coat and endosperm epidermis of Early Hegari 6 days after pollination. SC, seed coat; EE, endosperm epidermis. 216 ×.
Fig. 5 (top, right): Transection of Martin 30 days after pollination showing pericarp and portion of endosperm. P, pericarp; SC, seed coat; AL, aleurone. 56 ×.
Fig. 6 (bottom): Transection of crown region of Early Hegari 30 days after pollination showing pericarp and pigmented seed coat. EP, pericarp epidermis; M, mesocarp; CC, cross cell layer; TB, tube cell layer; SC, seed coat. 50 ×.

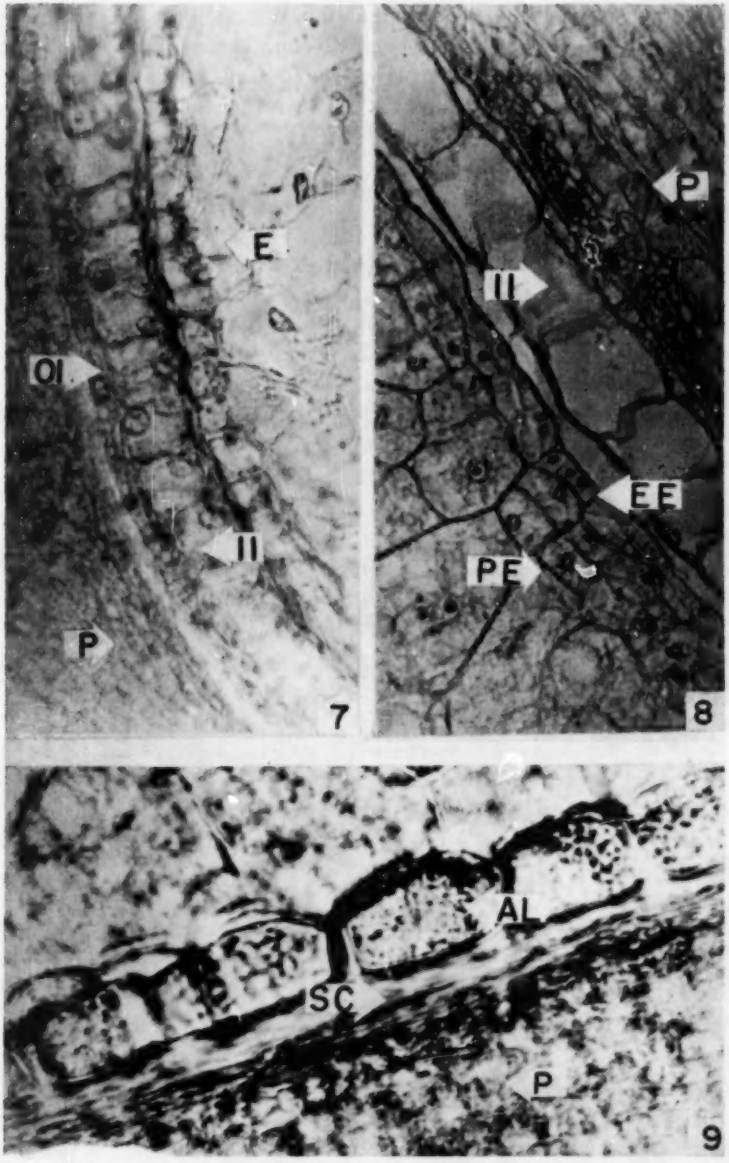
The seed coat is composed of a cutinous membrane and in some varieties there is a thicker layer of cellular remains. The pericarp is composed of four distinct layers (Fig. 6): the epidermis, *EP;* the mesocarp, *M;* the cross cell layer, *CC;* and the tube cell layer, *TB.*

General Development of the Kernel

At pollination (Fig. 2), the immature structures of the kernel are the ovule and the ovary wall. The four layers comprising the pericarp are all present in the ovary wall at the time of fertilization and remain until maturity. The ovary wall, which develops into the mature pericarp, completely encloses the ovule, which becomes the mature seed. The nucellus or nutritive tissue of the ovule contains an embryo sac where the endosperm and embryo are formed. The nucellus is covered by two integuments separated by a thin layer of cutin, which develops into the seed coat. With a few exceptions, the integuments disappear before maturity, leaving the intervening cutinous membrane next to the pericarp. Upon fertilization, the embryo and the endosperm are formed in the embryo sac. Subsequent development results in an embryo of highly specialized cells, and in an endosperm of storage cells.

Endosperm Development

Early Growth. Material collected 3 days after fertilization showed that there were cellular differences in the endosperm. At the margin, a single layer of cells of uniform size was distinguishable. The cells in this outer layer had dense cytoplasm and large nuclei but were much smaller than other endosperm cells. This outer layer became more prominent at 6 days (Fig. 4) as more cell divisions occurred. The cells divided in two planes, adding to the circumference of the endosperm considerably and to the interior of the endosperm. This resulted in an outer layer of cells distinct from the other endosperm cells. As these cells matured, their walls became thicker. Staining with Sudan IV showed that oil already had been stored at this early stage. These cells, covering most of the endosperm and embryo, formed an epidermal layer which later, after physiological changes, became the aleurone. However, the outermost cells at the base of the endosperm followed a different pattern of development (Fig. 10). Instead of dividing, these basal cells elongated and the cytoplasm within became disorganized. These cells were only one layer thick but remained in continuity with the cells in the outer layer of the endosperm. The growth of these cells was similar to the growth of the basal endosperm in corn (6). Although the endosperm below the embryo was in close



Figs. 7, 8, and 9. Fig. 7 (top, left): Transection near crown region of Combine Kafir 54T at 9 days. P, pericarp; OI, outer integument; II, inner integument; E, endosperm. 200 ×.

Fig. 8 (top, right): Transection of Combine Kafir 60, nine days after pollination showing empty cells of seed coat and formation of peripheral endosperm. P, pericarp; II, inner integument; EE, endosperm epidermis; PE, peripheral endosperm. 199.5 ×.

Fig. 9 (bottom): Transection of seed coat and aleurone of Martin 21 days after pollination. P, pericarp; SC, seed coat; AL, aleurone. 322 ×.

contact with the seed coat, the rest of the basal endosperm was separated from the seed coat. Since cell division ceased in the basal endosperm, a space was left as the pericarp and seed coat were pushed out

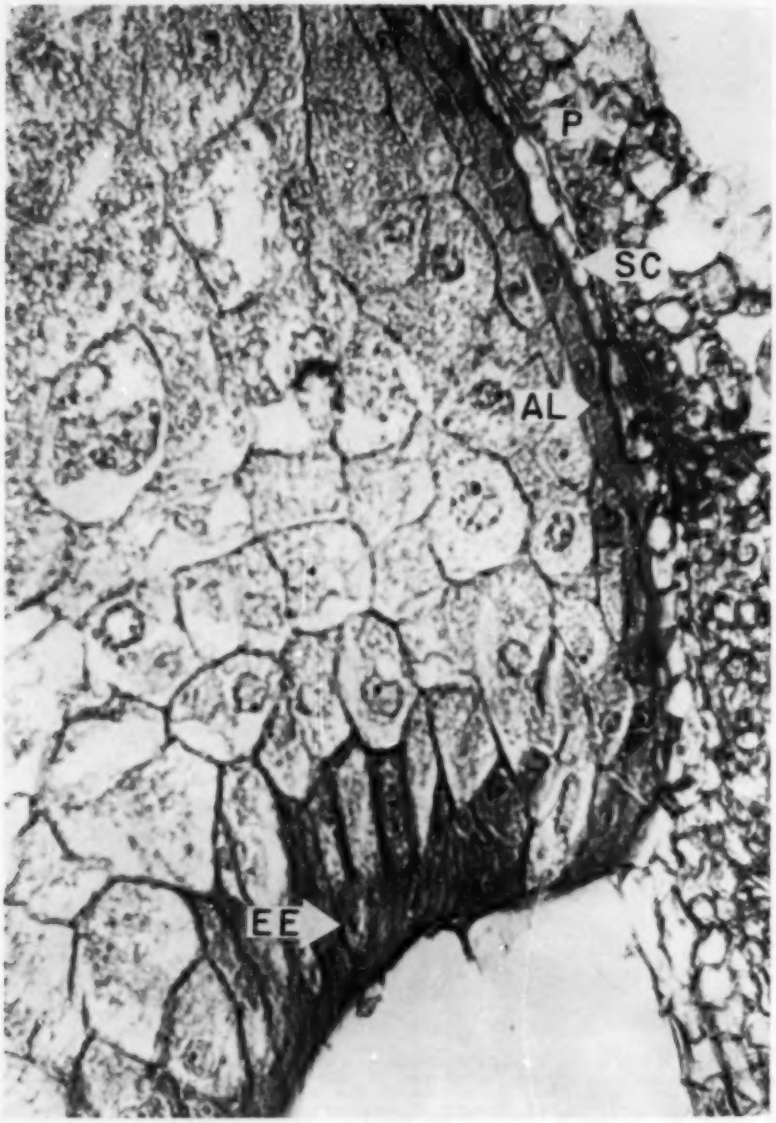


Fig. 10. Longisection at basal region of endosperm. P, pericarp; SC, seed coat; AL, aleurone; EE, endosperm epidermis, 277 ×.

by the growth in the rest of the kernel (Fig. 3). This space increased as the kernel matured.

At first, the subepidermal cells were irregular in shape and their cytoplasm content was low. As more cells were laid down by the epidermis, the subepidermal region was filled with smaller cells of regular shape arranged in file-like order (Fig. 8). This addition to the endosperm occurred at the same time that oil was being deposited in the epidermis, so that the content of oil in the subepidermal cells was greater than that in the floury endosperm. Cell divisions continued in this subepidermal region as starch was deposited in the other endosperm cells. Starch deposition, which began in the crown region, continued fairly rapidly so that two-thirds of all the endosperm cells contained starch 9 days after fertilization (Fig. 3).

During this early period of endosperm growth the original nutritive tissue of the ovule, the nucellus, had been digested and rapidly replaced by the endosperm. The latter tissue expanded from the basal region, enveloping the embryo and crushing the nucellus in the crown region. This initial growth of the endosperm was due to enlargement of the oldest endosperm cells as well as cell divisions in the subepidermal or peripheral region. These morphological developments occupied less than one-sixth of the total growth period of the endosperm. All of the types of endosperm cells present at maturity were laid down at this early stage of growth.

Maturation. The outermost layer or epidermis was the first part of the endosperm to mature. After cell division had ceased and cell walls had become thickened, this layer was distinguished by oil and protein deposits (Fig. 9). Except in the basal region, this layer, the aleurone, eventually enclosed the endosperm and the embryo.

Physiological changes in the central endosperm cells were as pronounced as in the epidermis. Starch was deposited in most of the endosperm cells except the subepidermal layer by the time cell division stopped in the epidermis. The nuclei of the endosperm cells, once prominent, were difficult to find in mature cells. Starch granules took the place of the cytoplasm, which was forced into a thin film around the granules and along the cell walls, giving the appearance of a protein matrix found in other grains such as corn (3). The endosperm cells in the central basal region were the last to be filled with starch. Although they were large and morphologically mature, starch deposition continued until the absorbing outer layer below was no longer functional.

Cell division continued in the subepidermal layer or peripheral endosperm after cell division stopped elsewhere, and the cytoplasm

remained more dense. Subsequent divisions in this region beneath the aleurone resulted in one or two concentric peripheral layers of flattened cells. In these immature cells of dense cytoplasm only a few starch granules were formed. The dense cytoplasm renders the peripheral layer conspicuous in stained sections. Cell division occurred last in the crown region, resulting in more peripheral cells in that region. More divisions in the region near the base of the kernel added to the number of peripheral cells in that region also.

Cell division ceased in the peripheral layer after starch was deposited and the protein network was formed. By this time the kernel had reached its maximum size and the arrangement of the surrounding mature cells left little room for growth of immature peripheral cells. Cessation of growth in the endosperm was brought about by changes in the basal endosperm and pericarp. The cells in the basal endosperm had elongated rapidly with their long axis at right angles to the surface of the endosperm. As their cytoplasm became discontinuous or vacuolated, nuclei became distorted and all the cells took on a "streaked" appearance (5). This was evident in unstained as well as stained sections. As physiological maturity approached, these basal endosperm cells were not crushed as they are in the corn kernel (6).

This general pattern of development was identical in all varieties studied, although in Early Hegari the first stages of development were complete in less time.

Seed Coat Development

Varietal differences were noted in seed coat development where they were lacking in endosperm development. At maturity, the seed coat was most conspicuous in Early Hegari. It was partially cellular in Combine Kafir 54T, but only a cutinous membrane was present in other varieties. Artschwager (1) referred to the seed coat as the fused pericarp and testa and described the latter as a layer of yellow or brown cells. Previous work (2, 8) described the seed coat in the mature kernel as remains of the nucellus. However, in the present investigation the origin of the seed coat was traced to the integuments alone. The origin of the seed coat in corn was also traced to the integument by Johann (4).

General Development. Two integuments were present at the time of pollination (Fig. 2); the inner one was two layers thick at the micropyle of the ovule, but the outer one was several layers thick except at the hilum or place of attachment to the ovary wall. Both integuments were discontinuous at the hilum where there was direct contact between the nucellus and ovary wall. After staining with Sudan IV, a

very thin layer of cutin was evident between the nucellus and the inner integument (Fig. 2). This cutinous layer was most prominent in the micropylar region.

Three days after pollination the outer integument had been crushed between the inner integument and the pericarp and had disintegrated rapidly, so that it was practically absorbed by the sixth day. This left the inner integument with its cutinous covering in direct contact with the tube cells of the pericarp. More cutin was deposited as the nucellus was digested. In the crown region, the inner integument consisted of large cells which were elongated at right angles to the surface of the kernel. At the 9-day stage (Fig. 8) these cells were prominent but they were crushed later like the outer integument. Until this time seed coat development was identical in all varieties studied. In most of the varieties, material collected 21 days after pollination showed little cellular tissue between the endosperm and pericarp (Figs. 5 and 9); exceptions were Early Hegari and Combine Kafir 54T.

Varietal Differences. The inner integument of Early Hegari grain sorghum was well developed before pollination; its cells contained an orange pigment which appeared to be granular in some cells. The outer integument disappeared, as described previously, as the endosperm grew, while inner integument cells increased in size but not in number (Fig. 4). As the cells enlarged they became tightly pressed one against the other, resulting in disruption of cell walls (Fig. 6). Expansion of cells was greatest in the crown region. As more pigment was produced and cell walls disintegrated, cellular configuration gave way to a continuous layer. By the time the kernel was mature all cell walls had disappeared, although the orange pigment in the basal region remained, broken into original cell shapes. Over the crown the pigment formed a solid and thicker layer, whereas over the face of the germ it was only one cell thick.

Among the varieties studied here, Early Hegari was the only one with pigment in the seed coat. Combine Kafir 54T had a nonpigmented layer which was partially cellular (Fig. 7). In this variety, square-shaped cells of the integument had lost their cell contents 21 days after pollination. Except in the crown region, pressure of the expanding endosperm gradually flattened the empty cells by collapsing their radial walls. Some cells, however, remained intact in that region. In other regions of the kernel, cellular material was absorbed so that at maturity it was totally absent. However, the cutinous membrane formed earlier was continuous with cellular remains in the crown region. Therefore, in all varieties there was a seed coat laid down between the endosperm and the pericarp.

Pericarp Development

Cells did not divide in the ovary wall or pericarp after pollination. Growth in this part of the kernel was confined to enlargement of cells already present. The configuration of cells of the pericarp was changed by pressure from the expanding seed beneath.

At the time of pollination the ovary was covered with a layer of cutin which extended up the style and down the stalk of the kernel. The epidermis beneath this layer of cutin was continuous except at the style and stalk. Immediately after pollination, as the style withered, more cutin was deposited in the stylar region and over the surface of the kernel. Epidermal cells beneath this layer of cutin began to lose their cell contents after an initial period of wall thickening. The walls of the next layer of cells (the outermost mesocarp) remained relatively thin, so that these cells were stretched and compressed as growth continued. The central mesocarp was made up of larger cells which were filled with small starch granules (Figs. 5 and 6). In Texioca this starch was of the nonwaxy type, whereas the larger granules in the endosperm were of the waxy type. Starch deposition continued in all cells of the mesocarp while the cells continued to grow in size. This growth continued until about the third week after pollination. About this time heavy thickenings appeared in the cells in the basal region of the pericarp. This occurred in cells surrounding conducting cells leading from the stalk. Eventually this was followed by formation of a hilar layer composed of suberin (Fig. 1), located below the specialized basal cells of the endosperm.

The tube and cross cells at the interior surface of the pericarp continued to lengthen and their cell walls became thicker as growth subsided. Pressure of the expanding endosperm did not cause these cells to collapse as did the cells in the outer mesocarp. Cytoplasm disappeared early from these cells as it had in the epidermal cells of the pericarp. Thickening of cell walls prevented these layers from collapsing as did many of the cells in the mesocarp. Also, starch granules prevented the complete collapse of the mesocarp. Maximum size of the kernel was reached about 3 weeks after pollination. The pericarp was fully expanded by this time and thereafter as its cells began to die, the endosperm and germ represented an increasing proportion of the volume of the kernel.

Discussion

Softening of the kernel and its separation into various structures present problems to the processor. In preparing the grain for milling, returning the proper content of moisture to the kernel is of primary

importance. Water absorption in the grain sorghum kernel is controlled by structural barriers. Cell wall thickening, as well as development of the seed coat, slows down the passage of water through the pericarp. Formation of the hilar layer also affects the supply of moisture to the endosperm and germ. Breeding investigations have proved that the cellular seed coat can be reduced to a cutinous layer. Exact information regarding the development of structures such as that of the seed coat should promote interest in further genetic modification of kernel structure.

Improvement of mechanical separation depends in part on breaking the cellular structures which have been revealed by these microscopic studies. Knowledge of the relationships of one structure to another should throw light on problems of separation. For example, different endosperm structure may account for differences in starch removal between grain sorghum and corn. An account of the peripheral endosperm layer in grain sorghum as it affects milling will be discussed in a subsequent publication.

Acknowledgments

The author wishes to acknowledge the suggestions and advice of Dr. S. A. Watson, under whose direction this investigation was accomplished.

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THE CONCENTRATION OF A FACTOR IN SOFT WHEAT FLOURS AFFECTING COOKIE QUALITY[1]

WILLIAM T. YAMAZAKI[2]

ABSTRACT

Procedures are described for the fractionation and reconstitution of soft wheat flours, and for the purification of the starch tailings fraction. This fraction had a deleterious effect on cookie spread of reconstituted flours. Purification of this fraction (which consisted principally of removal of granular starch) resulted in progressively greater ability to hydrate and increasingly detrimental effect on cookie spread. The purified starch tailings were very hydrophilic and significantly decreased the spread when added in small quantities to a control flour, their effect being proportional to the quantity added. Additions of similar hydrophilic materials to flours also decreased spread to varying degrees, indicating that the effect of the tailings was perhaps due in great part to their physical rather than chemical properties.

Straight-grade flours from harder wheat varieties appeared to contain more of this purified starch tailings component than those of softer varieties, suggesting that varietal differences in cookie baking ability may, at least in part, be attributable to quantitative differences in this fraction. A study of individual mill streams showed that lower-grade flours which gave poorer cookies were also richer in this component.

The purified tailings consist for the most part of endosperm cell walls, with some aleurone and bran. They are relatively low in starch and nitrogen and rich in pentosans. However, no definite relationship could be established between chemical composition and cookie spread.

Research concerned with the concentration of flour components contributing to bread quality has been carried out by several workers, among whom Morea (7), Harris (6), Sandstedt, Jolitz, and Blish (8), and Aitken and Geddes (1, 2) may be mentioned. They used the technics of fractionation and reconstitution, procedures further developed by Finney (4), who was the first to recover the water-solubles. He found that while gluten content and quality had important bearings on bread quality, the water-soluble fraction was also a consideration, especially among spring wheats.

Few references have been found in the literature relative to similar studies on soft wheat quality. A preliminary study was made by Yamazaki (9), who applied the methods of Finney, with modifications, to soft wheat flours.

He found that satisfactory bread could be baked from soft wheat flour fractions using as "flour" a dry blend of appropriate quantities of the fractions. However, before suitable cookies could be baked from the same fractions, it was necessary to form a dough, mix, dry,

[1] Manuscript received August 11, 1954. Cooperative investigation between the Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station, Wooster, Ohio.

[2] Associate Chemist, Soft Wheat Quality Laboratory, Wooster, Ohio.

grind, and rehydrate to form a "reconstituted flour." A number of variables related to the fractionation and reconstitution processes were studied.

This paper is an extension of the above work and reports the progress made in the concentration of a factor present in wheat flours which contributes to cookie quality.

Materials and Methods

Flours used included pure varietal composites of hard and soft wheats experimentally milled on the Allis and Buhler mills, and several commercial soft wheat flours. All were straight-grade and unbleached.

Analyses were made by methods commonly used in cereal laboratories. Cookies were baked by the micro baking procedure of Finney, Morris, and Yamazaki (5), wherein 40 g. of flour were used per batch. For reasons given elsewhere (5), cookie quality is expressed as the sum of average diameters of two cookies. Alkaline water-retention capacity (AWRC) values of various flour fractions were obtained according to the method of Yamazaki (10), using proportionately smaller quantities of sample for those fractions which had higher water-retentive properties.

The lyophilizing (freeze-drying) apparatus used in this work was an adaptation of one described by Westfall.[3] The condensing tank consisted of a brass cylinder with a baffle through the center which extended to within 2 inches of the bottom, as illustrated in Fig. 1. A tank of this design obviated the need for a secondary condenser. Nipples of the manifold were provided with large rubber stoppers against which jars containing samples were held until the atmospheric pressure was capable of maintaining a tight seal. The condenser tank was immersed in a bath of high flash-point kerosene and dry ice for rapid condensation of the sublimate.

Solutions and pastes for later lyophilization were frozen on the inside wall by pouring them into jars which were then slowly rotated on a horizontal axis while partially immersed in a mixture of 50% ethyl alcohol and dry ice. This procedure is referred to as "shelling."

Fractionation. Fractionations of flour using distilled water only were generally carried out in the following manner: To three parts of distilled water was added one part of flour by weight. This was stirred well and then centrifuged for 25 minutes at about 1000 r.c.f. The supernatant was collected, and to it was added the compressed flour dough. The dough was allowed to hydrate for about 30 minutes, fol-

[3] Private communication from Dr. R. J. Westfall, Sharp & Dohme, Inc., Philadelphia, Pa.

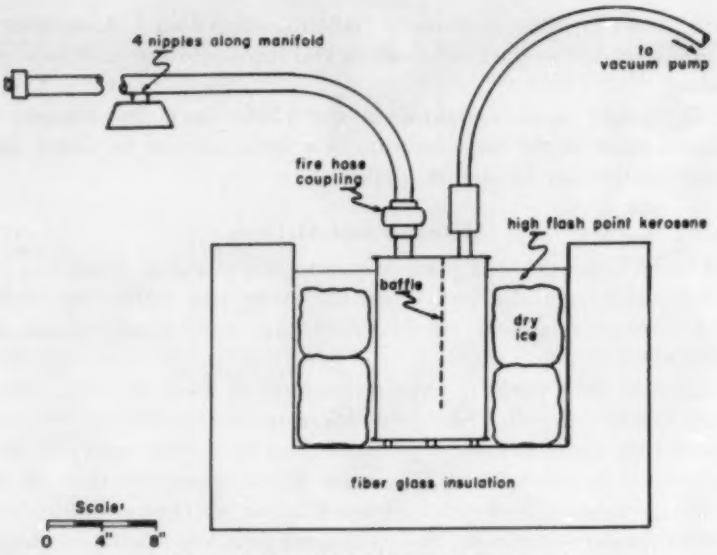

Fig. 1. Diagram of lyophilizing apparatus.

lowed by gentle kneading in the supernatant liquid until separation of starch from gluten was effected. This step required great care to prevent disintegration of the gluten in cetrain cases. The gluten was washed thoroughly with distilled water and then lyophilized after slices were frozen in jars. The starch slurry (containing gluten wash water) was centrifuged. The supernatant liquid was then shelled and lyophilized (water-soluble fraction) while the starch sediment was made into a paste and also shelled and lyophilized.

Reconstitution. A flour reconstitution method found to give satisfactory cookies follows: The desired proportions of fractions were weighed and sufficient water was added to form a slack dough. This was mixed to optimum gluten development, placed in a jar, and lyophilized without prior freezing. This permitted the expansion of the dough and subsequent drying in a porous state. The dried dough was ground and rehydrated in a humidifying cabinet to about 14% moisture.

After experiments showed the importance of the starch tailings fraction in cookie quality, separations were made of prime starch and crude tailings from crude starch on a routine basis. This was accomplished by centrifuging a thin slurry of crude starch. The sediment consisted of two layers, an upper viscous (starch tailings) stratum and a lower, rather dry, well-packed, white (prime starch) layer which could be divided mechanically.

Crude Starch Purification. The procedure used for the purification of the crude starch tailings was essentially as follows: A thin slurry of the crude starch tailings fraction was made and mixed in a Waring Blendor for 2 minutes at high speed. It was then wet-sieved through a vibrating 325-mesh nylon sieve, and the material remaining on the sieve retained. The material passing through was collected and re-sieved a second and third time. The combined overs were then made into a paste and lyophilized. In some cases a further separation was made by centrifuging the slurry containing the overs of the sieve. This resulted in the formation of several layers in the sediment, a white translucent gel-like upper sediment blending into a tan- to brown-colored lower layer. A mechanical separation was made, retaining the white upper layer which was lyophilized.

Alkaline Fractionation. The aforementioned gluten-kneading method of flour fractionation is rather tedious and time-consuming. In cases where recoveries of gluten and water-solubles were not required, a more rapid method was adopted which made use of a dilute solution of sodium hydroxide following the procedure described by Dimler, Davis, Rist, and Hilbert (3). The usual procedure was to take one part flour and eight parts 0.01 *N* sodium hydroxide solution, mix well, and centrifuge. A second treatment with two parts 0.01 *N* sodium hydroxide solution was given to the residue. Centrifugation of this suspension resulted in the formation of two layers in the residue, which could be separated mechanically. The upper alkaline crude tailings layer was then neutralized. Tests conducted on the purified starch tailings indicated that the quality of the product was the same as that produced by the longer procedure.

Results

Application of the AWRC Test to Fractionation. On the basis of previous results (10, 11), it appeared that cookie quality is an inverse function of the hydration or water-retention characteristics of soft wheat flours. Hydration characteristics were therefore the criterion used in this study for the concentration of factors affecting cookie quality.

The AWRC test was thus applied to various flour fractions in order to evaluate them for possible effects on cookie spread. Although the water retentions of the glutens from different varieties were relatively high, they did not correlate with the cookie qualities of the flours. On the other hand, the crude starch fractions gave values which, while low, appeared to be related to varietal cookie spread.

Further separations into prime starch and crude starch tailings were therefore made with the starches from several varieties.

Effect of Starch Tailings on Cookie Spread. AWRC values of the prime starches showed no significant varietal differences, but those for the crude starch tailings were relatively high (higher than gluten in most cases) and appeared to be inversely correlated with varietal cookie baking properties. To determine whether these results were associated with quality – as Yamazaki (11) has reported for AWRC, bread dough absorption, and cookie spread for soft wheat flours – reconstituted flours were prepared from fractions of several varieties in such a way that the crude starch tailings fractions were present at three levels; zero tailings, normal quantity, and twice the normal quantity. Cookies baked from these flours are shown in Fig. 2. The quantity of

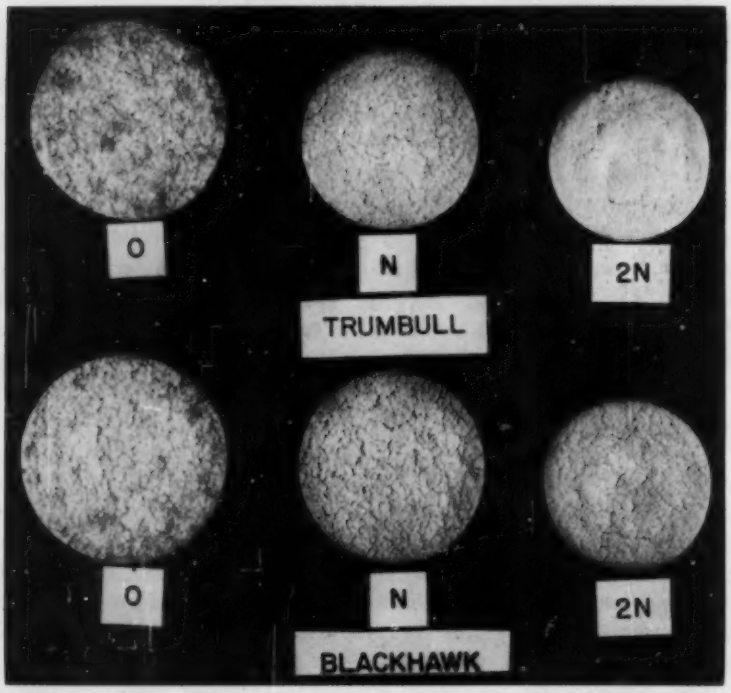

Fig. 2. Effect of crude starch tailings levels on the spread of cookies from reconstituted flours of Trumbull and Blackhawk varieties, respectively. Quantities used are: 0, no tailings; N, normal quantity; 2N, twice the normal quantity.

the crude tailings present had an important bearing on the spread of cookies. However, when tailings were omitted, the spread of cookies still appeared to be a varietal characteristic, that is, the diameters of

cookies from fractions of the Trumbull variety were less than those of the corresponding cookies from Blackhawk. Trumbull is known to have fair cookie baking quality while Blackhawk is superior in this respect. Whether these differences without starch tailings were due to the presence of another component or to tailings impurities in the respective crude glutens has not been determined.

Purification of Starch Tailings. Because of the apparent importance of the crude starch tailings fraction in cookie quality, further physical separations of this fraction were made in an attempt to concentrate the factor present. These separations consisted primarily of the removal of granular starch, either by centrifugation followed by mechanical separation of tailings from starch, or by one or more wet-sieving operations, or both. Products obtained by these operations are called "purified tailings."

At this point a change was made in the method of testing the effect of a flour fraction on cookie quality. Rather than reconstitute a flour with varying quantities of the material under test, small amounts of fractions were added to a normal flour used as a control. This procedure was practicable when the purified tailings were tested, owing to their hydrophilic nature and the considerable effect of small additions on cookie spread.

Under this method of testing, progressively greater effects on cookie spread were noted with "purification" of starch tailings. An example of results obtained by means of an alkaline fractionation followed by wet-sieving is presented in Table I.

TABLE I

EFFECT OF FLOUR FRACTIONATION ON YIELD, ALKALINE WATER RETENTION CAPACITY, AND COOKIE SPREAD WHEN ADDED TO A CONTROL FLOUR

	Yield on Flour Basis	AWRC	Cookie[a] Spread
	%	%	*cm.*
Original flour	100	47.7	17.1
Residue after first stage of alkaline fractionation	34	86.5	17.1
Crude tailings after second stage of alkaline fractionation	5	269	16.7
Purified tailings	0.6	1060	15.4

[a] Sum of diameters of two cookies obtained when 1 g. of fraction was blended with 39 g. control flour.

The spread-depressing effect of the purified tailings when added to a control flour was nearly proportional to the quantity present.

Figure 3 is a scattergram bearing on this relationship. With the addition of 5% of purified tailings to the control flour, a spread of 12.5 cm. was obtained for two cookies. This was a reduction of 4.7 cm. from the 17.2-cm. diameter of the control flour, and the diameter of each cookie actually was not much greater than the cutter itself, twice the diameter of which was 12.3 cm.

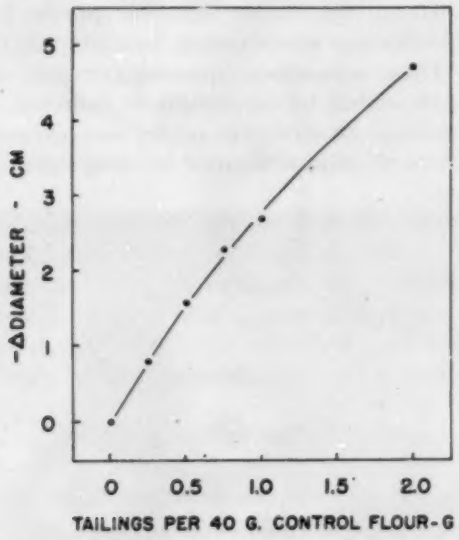

Fig. 3. Scattergram showing effect on decrease in cookie diameter of additions of purified tailings to a control flour.

To determine whether this deleterious effect on cookie spread caused by the purified tailings was a physical property or was due to chemical composition, various hydrophilic systems were added to a control flour and their effects on cookie spread noted. Table II presents AWRC and cookie data for a few of the materials used. Such substances as lyophilized agar gel and purified tailings (alkaline fractionation) had considerable detrimental effects. Products as diverse as xylan, lyophilized hydroxyethyl cellulose, boiled starch, crude tailings treated with hydrochloric acid and amylase, and lyophilized gelatin influenced the spread in varying degrees. More will be said later concerning these results.

Variety Study. One of the questions which arose was the possible relation of the purified tailings fraction to varietal differences in cookie baking quality. To investigate this problem, fractionations were made of flours from Pawnee (hard), Purkof (semihard), and Black-

TABLE II

ALKALINE WATER RETENTION CAPACITY OF VARIOUS HYDROPHILIC MATERIALS AND THEIR EFFECT ON COOKIES WHEN ADDED TO A CONTROL FLOUR

	AWRC	Cookie Dough[a] Absorption	Cookie[b] Spread
	%	*ml.*	*cm.*
1. Agar, granular	790	10.3	16.4
2. Agar gel, lyophilized	Soft	12.0	14.5
3. Gelatin, granular	820	10.2	16.8
4. Gelatin gel, lyophilized	Sol.	10.9	16.3
5. Gluten, Blackhawk	137	10.6	16.7
6. Gluten, Pawnee	132	10.6	16.5
7. Hydroxyethyl cellulose	1030	10.0	16.4
8. Hydroxyethyl cellulose, lyoph.	960	10.2	15.6
9. Starch, prime	55	9.8	17.1
10. Starch, prime, ball milled	430	10.2	16.1
11. Starch, prime, boiled	980	10.8	15.2
12. Tailings, crude, boiled	...	11.0	15.1
13. Tailings, crude, HCl-treated	...	10.8	15.9
14. Tailings, crude, diastase-treated	...	11.3	15.8
15. Tailings, purified, alk. frac.	1290	11.8	14.1
16. Xylan, acid-precipitated	Soft	10.6	15.3

[a] Cookie dough absorption for optimum handling consistency when 1 g. of material was blended with 39 g. control flour.
[b] Sum of diameters of two cookies obtained when 1 g. of material was blended with 39 g. control flour.

hawk (soft) wheats, using the gluten-kneading method to obtain the starch fractions. The crude tailings were purified by washing out the starch by wet-sieving. Data for these separations and cookie results are presented in Table III.

There appeared to be an inverse relationship between the total quantity of material held by the sieves and the cookie spreads of these flours obtained from different varieties of wheat.

Mill Stream Study. This relationship was further tested with individual mill streams from a Buhler milling of Thorne (soft) wheat. After the usual analytical and baking tests on the streams (Table IV), each was fractionated by the gluten-kneading method and the crude starches purified as in the variety tests. The first and third breaks and first and third reductions were separated in this manner, and the yields and analytical data for these fractionations are presented in Table V. Here again there appeared to be an inverse relationship between the quantity of purified tailings and cookie spread (compare last columns, Table IV and Table V).

The purified tailings obtained in all cases were not identical in appearance. Materials from the lower grades of flour contained rela-

TABLE III

YIELD, ALKALINE WATER RETENTION CAPACITY, AND COOKIE DATA FOR FRACTIONS OBTAINED IN VARIETY STUDY

Variety	Fraction	Yield Based on Flour	AWRC	Cookie [a] Dough Absorption	Cookie [b] Spread
		%	%	*ml.*	*cm.*
Pawnee	Gluten	20.4	122	10.6	16.9
Purkof		15.5	122	10.6	16.8
Blackhawk		12.8	124	10.5	17.0
Pawnee	Crude starch	74.0	...	...	...
Purkof		78.1	...	...	...
Blackhawk		79.9	...	...	...
Pawnee	Water-solubles	4.7	...	10.2	17.0
Purkof		3.8	...	10.5	16.4
Blackhawk		5.0	...	10.2	17.1
Pawnee	Crude tailings	10.7	238	10.4	16.4
Purkof		11.5	214	10.4	16.6
Blackhawk		11.3	147	10.4	16.6
Pawnee	Over 25 std. sieve	1.07	880	12.2	15.1
Purkof		1.22	1140	12.4	14.8
Blackhawk		0.97	860	12.2	15.3
Pawnee	Over 325 sieve	0.77	1070	12.4	15.0
Purkof		0.33	1680	13.0	14.4
Blackhawk		0.24	1400	12.7	14.7
Pawnee	Total over sieves	1.84			
Purkof		1.55			
Blackhawk		1.21			

		Cookie Spread [c]
		cm.
Pawnee	Original flour	15.5
Purkof		17.1
Blackhawk		18.2

[a] Cookie dough absorption for optimum handling consistency when 1 g. of fraction was blended with 39 g. control flour.
[b] Sum of diameters of two cookies obtained when 1 g. of fraction was blended with 39 g. control flour.
[c] Sum of diameters of two cookies.

TABLE IV

YIELD AND COOKIE DATA FOR BUHLER MILL STREAMS OF THORNE WHEAT

Stream	Yield on Wheat Basis	Cookie Dough Absorption [a]	Cookie Spread [b]
	%	*ml.*	*cm.*
First break	7.6	8.9	17.8
Third break	3.1	9.8	16.2
First reduction	26.9	9.3	17.3
Third reduction	7.3	10.0	15.9

[a] Volume of water for optimum handling consistency using 40 g. flour.
[b] Sum of diameters of two cookies.

TABLE V

YIELD DATA FOR FRACTIONATION OF BUHLER MILL STREAMS OF THORNE WHEAT

Stream	Yield on Flour Basis[a]				
	Gluten	Starch	H_2O Sol.	Crude Tailings	Purified Tailings
	%	%	%	%	%
First break	12.4	83.2	4.3	16.1	0.91
Third break	28.2	67.3	4.5	11.1	2.23
First reduction	16.9	78.9	4.2	13.3	1.09
Third reduction	17.8	76.5	5.7	17.2	3.41

[a] 14% moisture basis.

tively large quantities of impurities which gave darker colors to the product. Therefore the recoveries were not considered to be quantitative but merely indicative of trends in the recoveries of the purified product. Considerable difficulty was encountered in attempting to separate the highly retentive white layer from impurities without alteration in the physical properties. It is believed, however, that the values presented give an indication of the relative quantities of the hydrophilic systems in the various mill streams. Similar large differences in physical properties of purified tailings were found in the variety study.

Properties of Purified Tailings. The purified tailings, after empirical separation of the translucent gel-like material from impurities and drying by lyophilization, was a white-to-yellowish feathery solid, capable of absorbing very large quantities of water (from 10 to 16 times its own weight as determined by the AWRC method). Under the microscope it appeared to consist for the most part of endosperm cell wall material, taking readily such cell wall stains as benzopurpurin, Congo red, and molybdenum blue. Various bran layers could also be seen in small quantities as impurities. Chemical analyses gave rather wide ranges for components. For example, total nitrogen varied from about 0.5 to 3% depending on the method of separation, starch from 7 to 23%, and pentosans from 30 to 65%. It was not possible to correlate the chemical composition of the purified tailings with their effects on cookie spread.

Discussion

Purified tailings obtained in the variety and mill stream fractionation experiments bore semiquantitative inverse relationships to the cookie baking potentialities of the flours used. In the variety experiment, the purified materials all had consistently high alkaline water-retentive values, sorbing from 10 to 16 times their weight of water,

and had similar detrimental effects on spread when blended with a control flour and baked into cookies. However, flours which baked into larger cookies gave lower yields of this fraction than those which baked into poorer cookies. The products obtained from concentration of tailings from the various mill streams were not as uniform. Those from the low-grade flours were lower in retentive capacity than those from high-grade flours, owing to heavier contamination by components having low hydration capacities, but their total yields were much greater.

The finding of a component of flour, the quantity of which is inversely related to cookie quality, together with the highly hydrophilic nature of the component, would appear to lend support to the idea that cookie quality may be expressed in terms of a flour-water relationship. It appears likely that there is a quantitative relationship between the hydrophilic component and cookie baking property, larger amounts tending to cause flours to bake smaller cookies. This component appears to be fibrous. At least, under the microscope, there seems to be a preponderance of cellulosic components such as bran, aleurone, and endosperm cell walls. Separation of purified tailings from a low-grade flour may be effected by centrifugation followed by mechanical removal of a translucent gel-like upper layer which is lacking in bran and low in aleurone. This fraction, rich in endosperm cell walls, is high in AWRC and has considerable effect on cookie spread when added in small quantities to a control flour. The dark-colored residue has more bran and aleurone, although quantities of endosperm cell walls are still in evidence. AWRC values are lower for this layer, and effect on spread is not so great. Thus, it would appear that the endosperm cell walls are a major factor. Treatment of tailings materials with agents that cause hydrolysis of starch and pentosans (such as hot hydrochloric acid or diastase, many preparations of which have pentosanase activity) decreases their effect on spread. This would indicate that there is perhaps a concentration of the less hydrophilic components (fats, less readily attacked cellulosic materials, etc.), or there are changes in the physical properties of the residue.

That the physical nature of the component is important is emphasized by the experiment in which several hydrophilic materials were added to a control flour. Among agar, gelatin, and hydroxyethyl cellulose, the lyophilization process appeared to cause relatively large differences in effect on spread. Ball milling or boiling of prime starch may be expected to decrease the degree of polymerization of the hexosan, and result in greater exposure of centers of attraction for water molecules.

It thus appears that the effect on cookie spread is primarily physical. It may well be that any component of flour which can sorb relatively large quantities of water will have a detrimental effect on cookie spread; and further, that the cookie baking potentialities of a flour are determined by the sum of the hydrophilic components, regardless of their chemical composition.

Acknowledgment

Acknowledgment is made of the valued assistance of Mr. Lloyd Moser in the work reported.

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FLOUR PARTICLE SIZE AND ITS RELATION TO WHEAT VARIETY, LOCATION OF GROWTH, AND SOME WHEAT QUALITY VALUES[1]

R. H. HARRIS[2]

ABSTRACT

Fractions separated from experimentally milled hard red spring wheat flour according to particle size were influenced by the location of growth, and to a lesser extent by variety, for wheats found satisfactory in milling quality. All interactions involving the three sources of variation—varieties, locations, and sieve sizes—were very significant. Varieties which were difficult to mill showed very marked differences in their particle size distribution from varieties known to be satisfactory for milling. Six fractions from one hard red spring wheat flour differed in loaf volume, the best loaves occurring with the intermediate particle sizes, and the smallest loaf with the smallest size of particle. The fraction yielding the smallest loaf had abnormal mixing properties. Flour ash content varied inversely with particle size.

Several workers (1, 4, 5, 8, 10) have recognized that flour particles vary in size and differ in chemical and physical properties and that baking quality is affected, to some extent at least, by flour granulation.

Shollenberger (10) showed that wheat endosperm particles do not invariably correspond to the apertures of the flour cloth through which they pass during sieving. LeClerc, Wessling, Bailey, and Gordon (6) reported that flour sifted through fine silk bolting cloth was slightly inferior in baking quality to that sieved through coarser cloth, but flour sifted through cloth of intermediate size was best of all. Other workers (5, 7) also found that the coarsest and finest flour particles exerted a detrimental effect on flour quality in general. Apparently, finer flour particles had a lower protein content than those of larger size (9, 12).

Wichser, Shellenberger, and Pence (15) discussed the difficulty of separating flour into well-defined fractions by the use of silk flour cloths because of limitations of mesh fineness and the tendency of the particles to agglomerate. These workers made a complete fractionation of flour into several particle size groups with the Ro-Tap shaker and metal sieves and determined their physical and chemical properties and baking qualities. Their results confirmed those formerly obtained by more empirical methods and added new evidence about the relation between flour particle size and the characteristics of flour in gen-

[1] Manuscript received May 27, 1954. Published with the approval of the Director, North Dakota Agricultural Experiment Station.
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eral. Wichser and Shellenberger (13) showed that hard red spring, hard red winter, and soft wheat flour differed in their granulation curves. There was an inverse relation between particle size and ash and protein content for the hard wheat flours, but a reversal of the ash relationship was obtained with the soft wheat flour. Method of preparation of the wheat for milling was without effect upon flour characteristics (14).

In view of the interesting results of Wichser *et al.* it was decided to study the particle size distribution of experimentally milled hard red spring wheat flour and assess the value of this procedure for detecting milling differences between wheat varieties, especially new hybrids under investigation.

Materials and Methods

The flours were long patents experimentally milled in the Allis from sound samples of hard red spring wheat grown in 1953, principally in North Dakota. A composite flour milled from several standard varieties grown in the state was employed in determining the time of optimum sieving and for fractionation. For ascertaining the influences of variety and location of growth upon particle size distribution, six varieties grown at six locations were employed. These varieties and locations are identified in Table I, which shows the average test

TABLE I

SOME AVERAGE QUALITY VALUES FOR VARIETIES GROWN AT SIX STATIONS
(Arranged in order of decreasing flour yield)

Wheat Variety	Test Weight	Wheat Protein Content[a]	Total Flour Yield	Flour Ash[a]
	lb/bu	%	%	%
Selkirk	58.5	13.8	72.4	0.42
Rushmore	57.0	12.7	70.5	0.42
3880	59.3	14.0	70.2	0.42
Lee	57.6	13.1	69.3	0.43
Mida	54.3	11.9	66.3	0.43
Thatcher	53.6	12.3	66.1	0.45

[a] Expressed on 14.0% moisture basis.

weights, wheat protein contents, flour yields, and ash contents. Test weight and protein content were lower than usual, especially for varieties lacking in resistance to stem rust 15B. Three other varieties were examined; two grown at Fargo, and the other at Brookings, South Dakota. These wheats have not been approved for release. Wheat protein content, flour yield, and ash values for these three varieties follow:

	A	*B*	*C*
	%	%	%
Wheat protein content	13.6	12.2	11.3
Total flour yield	66.7	62.1	62.1
Flour ash	0.40	0.44	0.38

Methods. The sample preparation, milling, baking, and analytical methods used have been described (2, 3, 11). The particle size distribution of the flours was determined by using one Tyler sieve at a time

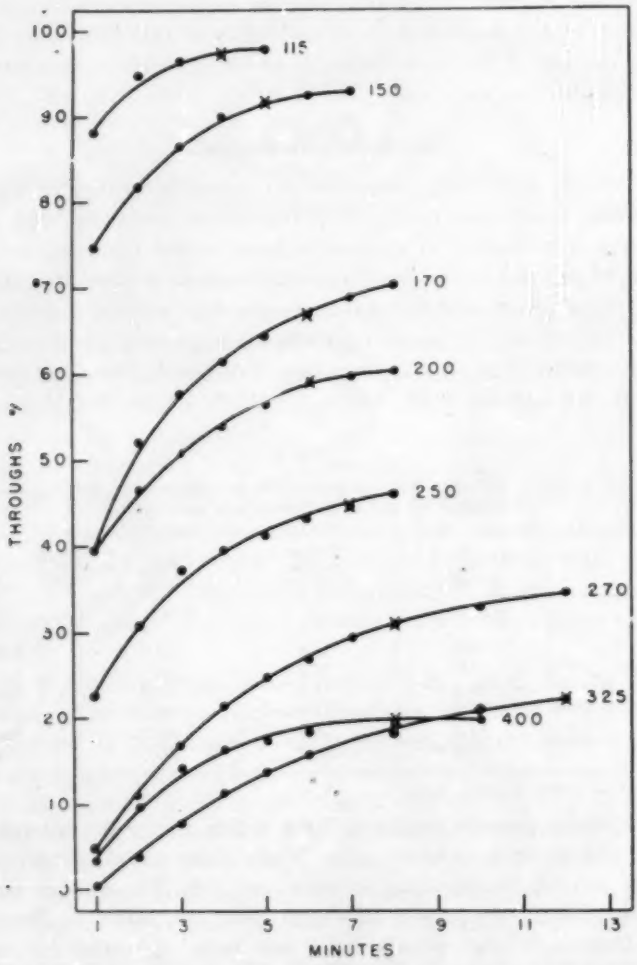

Fig. 1. Relation between minutes of sieving and material passing each testing sieve. Optimum time indicated for each curve. Sieve numbers refer to number of meshes per linear inch (Tyler).

in a Ro-Tap shaker.[3] This apparatus and its use has been discussed by Wichser, Shellenberger, and Pence (15). The sieve was placed over the bottom pan, which carried an under-sieve brush cleaner. Another small cloth cleaner was placed on the upper surface of the test sieve. Fifty grams of flour were distributed over the surface of the sieve and the weight of throughs was plotted against time of sieving, at intervals of 2 minutes. Optimum sieving time was chosen when the throughs approached a constant value. Additional sieving caused a slight gain, probably because of the effect of attrition. All sieves employed were handled in this manner; Fig. 1 shows the data obtained. The points at which the sieving was deemed complete for each mesh size are indicated. The optimum sieving time and particle size distribution differ from those of Wichser, Shellenberger, and Pence (15), who worked with commercial flours. This procedure was also used to compare the particle size distribution curves for the flour milled from wheats grown at different stations.

For the larger quantities needed in analyzing and baking the fractions it was necessary to modify the method as follows. Fifty grams of flour were sifted on the finest sieve (400-mesh) for the number of minutes required to approach a constant amount of throughs for that particular mesh, then the overs were removed and a fresh sample of the same flour was substituted. This procedure was repeated until a sufficient quantity of throughs was obtained. The next finer mesh was used for sifting the material retained on the first sieve, or overs, and this process was repeated for the succeeding coarser meshed sieves.

Results and Discussion

Flour Fractions. Table II shows the moisture, protein, and ash contents of the original and six flour fractions, with their baking and mixing properties. As suspected, moisture loss increased with the number of sievings, with a total loss of 2.3% between the original flour and the fractions passing 200- to 115-mesh. These fractions had the longest exposure to the laboratory atmosphere, which was not humidified. The more rapid initial losses would also be expected because of longer sieving time and higher flour moisture content.

As found by Wichser and Shellenberger for commercially milled flour, the protein content and loaf volume increased as flour particle size decreased, except for the fraction passing the 400-mesh sieve. This fraction was 1.6% lower in protein and 95 cc. lower in loaf volume

[3] "The profitable use of testing sieves," Catalog 53, pp. 20-22. W. S. Tyler Co., Cleveland, Ohio (1952).

TABLE II

ANALYTICAL AND MICRO BAKING DATA FOR THE ORIGINAL FLOUR AND SIX FRACTIONS PASSING INDICATED MESHES

Mesh No.	Moisture	Protein[a]	Ash[a]	Absorption	Loaf Volume	Crumb Color[b]	Grain and Texture[c]	Crust Color[d]	Symmetry[e]	Mixogram Type
	%	%	%	%	cc.					
Original flour	13.5	13.1	0.40	61.6	210	8.5	7.5	S	4.5 O	Medium
115	11.2	13.0	0.31	60.0	160	7.5 d	5.5 c	P	2.5 O	Medium
150	11.3	13.7	0.32	60.0	180	9.0	7.5	Sl P	4.0 O	Medium
200	11.2	14.7	0.35	60.4	185	9.0	7.5	S	4.0 O	Medium
250	11.4	15.8	0.38	61.2	210	9.0	7.5	S	4.5 O	Med. strong
270	11.7	16.7	0.47	62.0	240	9.0	6.5 O	S	4.5 O	Strong
400	12.0	11.5	0.60	64.0	115	6.0g&y	3.5 C	D	1.0 O	Med. weak

[a] Expressed on 14.0% moisture basis.
[b] Crumb color: d, dull; g, gray; y, yellow. Perfect score, 10.0.
[c] Grain and texture: c, close; O, open; C, coarse. Perfect score, 10.0.
[d] Crust color: P, pale; Sl, slightly; D, dull; S, satisfactory.
[e] Symmetry: O, overoxidized. Perfect score, 5.0.

than the original flour, and 5.2% and 125 cc. below the fraction passing the 270-mesh sieve. Flour absorption increased generally as particle size decreased, with the smallest fraction having the highest value in spite of its low protein content and baking quality. Ash content also varied inversely with particle size. Figure 2 represents the appearance

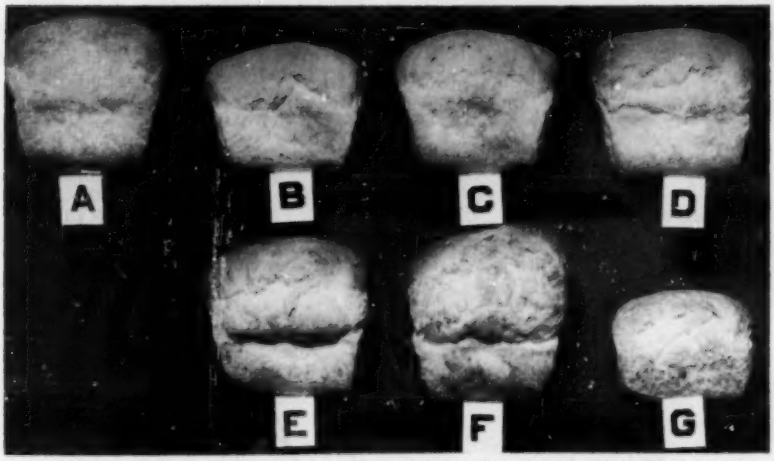

Fig. 2. Comparative loaves baked from the original and six flour fractions. Loaf A, original flour; B, through 115-mesh (105-125μ); C, through 150-mesh (74-105μ); D, through 200-mesh (61-74μ); E, through 250-mesh (53-61μ); F, through 270-mesh (38-53μ); G, through 400-mesh (0-38μ). Micron distribution according to Wichser, Shellenberger, and Pence (16).

of the loaves baked from the different flours. Loaves E and F were particularly good, while loaf G was markedly poor and unsatisfactory.

The mixing properties of the flours corresponded approximately to the protein content and loaf volume (Fig. 3). The "strength" of the

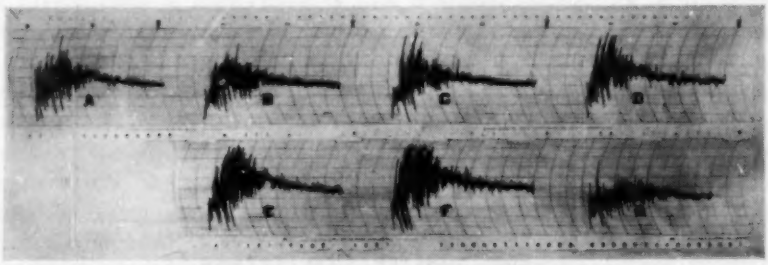

Fig. 3. Mixogram patterns of the original and six flour fractions. Mixogram A, original flour; B, through 115-mesh (105-125μ); C, through 150-mesh (74-105μ); D, through 200-mesh (61-74μ); E, through 250-mesh (53-61μ); F, through 270-mesh (38-53μ); G, through 400-mesh (0-38μ).

mixogram increased with decreasing particle size until 400-mesh was reached. This fraction produced the weakest and most unsatisfactory

curve of the group. Curves from the 250- and 270-mesh material (curves E and F) were slightly stronger than for the original unfractionated flour.

Evidently flour particles passing 250- to 270-mesh sieves (38–61μ) (15) are superior in general baking quality to those passing coarser or finer meshed sieves, as suggested by other workers (6, 15). It should be possible to alter and improve the baking quality of flours by judicious blending of fractions of suitable particle size and thus meet a wide variety of quality specifications.

Wheat Varieties. Figure 4 represents the particle size distribution

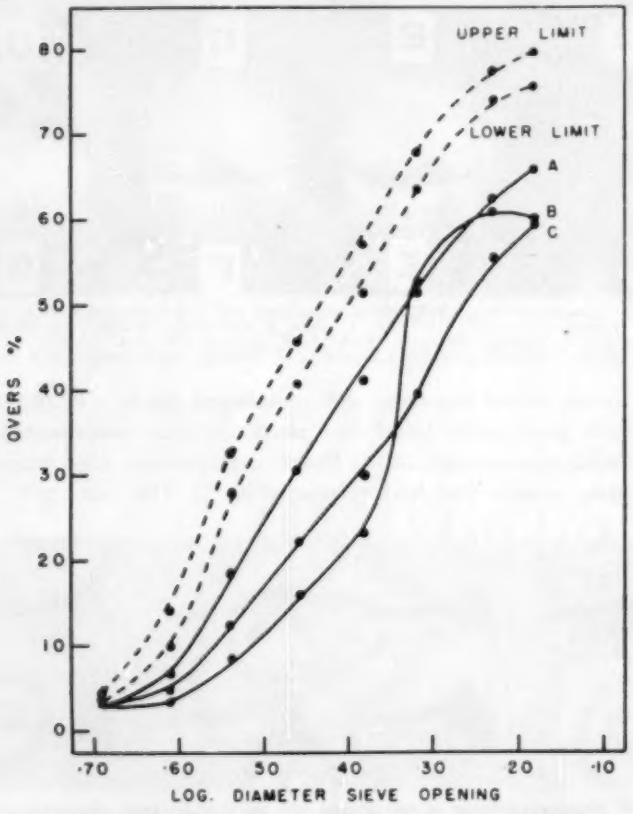

Fig. 4. Particle size distribution curves for different hard red spring wheat flours. Broken lines indicate distribution limits for six acceptable milling varieties. Unbroken lines show the distribution for new varieties not released and of doubtful milling quality.

for the varieties, using the logarithm of the diameter of the sieve aperture.[3] Six were classed as acceptable in milling quality, and

fell within the limits delineated by broken lines for their particle size distribution. Lee and Thatcher tended to attain the higher limit, while Selkirk and Rushmore were nearer the lower. Although the variability in this group is relatively small, it was highly significant (Table III), showing that even wheats judged satisfactory for milling

TABLE III

ANALYSIS OF VARIANCE OF SIEVING DATA FOR SIX VARIETIES OF SATISFACTORY MILLING QUALITY

Source of Variation	Degrees of Freedom	Variance[a]
Between varieties	5	72.2
Between stations	5	485.3
Between sieves	7	28736.6
Interactions:		
Varieties × stations	25	25.2
Varieties × sieves	35	9.1
Stations × sieves	35	20.3
Varieties × stations × sieves (error)	175	3.3
Total	287	

[a] All variances were significant at the 1% point.

by usual experimental and pilot mills differ in this characteristic. The remaining three varieties, A, B, and C, were lower in milling quality and their middlings more difficult to reduce. The particle size distribution of their flours was markedly different from the first group and showed that they were much less vitreous, confirming conclusions reached by means of conventional milling procedures and arousing suspicion regarding their milling quality. It is planned to separate fractions from these and compare their quality with that of fractions from a flour milled from a wheat satisfactory in milling behavior.

The flour yield did not seem to be related to the particle size distribution of the varieties. There was no significant difference in ash content among these varieties in spite of the significant variations in particle size distribution among them (Table III). Wheat protein content was also without effect on size distribution, and was not related to vitreousness, as judged by kernel appearance. Samples B and C were markedly lower in flour yield than the other varieties, corresponding to their flour particle size distribution. However, sample A had the same flour yield as acceptable varieties, although differing from them in particle size distribution and kernel appearance.

Growth Location. Figure 5 provides information on the influence of growth location on the flour particle size distribution of the six

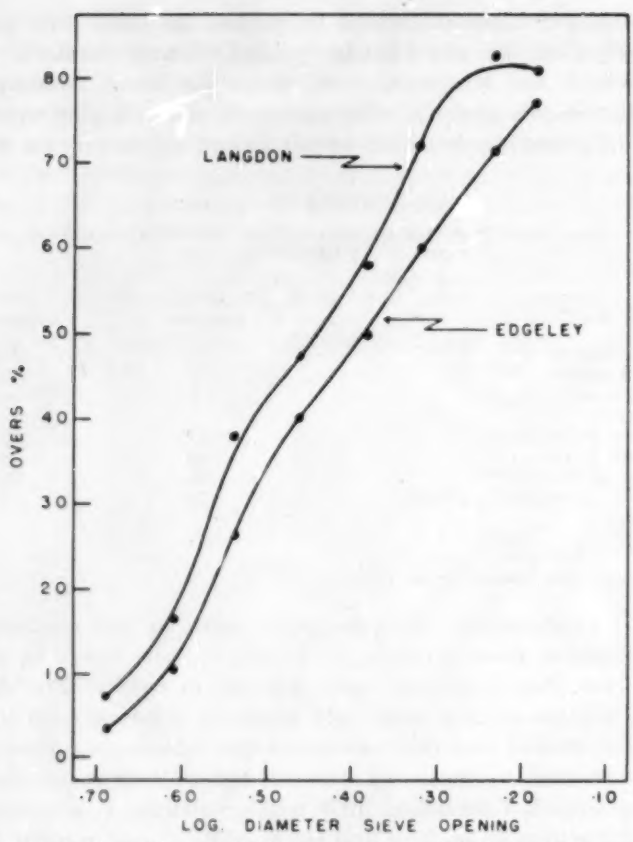

Fig. 3. Limits of particle size distribution for flours milled from wheats grown at six locations in North Dakota.

varieties. Although six locations were included in the study, only results from two locations, one representing the minimum and the other the maximum values, are shown for clarity. The differences due to locations were much greater than those due to varieties (Table III), implying that environment is more influential than variety on flour particle size distribution for these varieties of good milling quality. Dickinson and Langdon were quite similar, but significantly different from the mean of the other four stations, which resembled each other rather closely.

For the stations as for the varieties there was no evident correlation between flour yield and particle size distribution. Although flour ash content varied very significantly among the stations, it was not related to particle size.

The interactions in Table III show that wheat varieties are not similarly affected in flour particle size distribution by different areas in which they were produced, or by changes in sieve mesh. Another point, possibly of minor importance, is that the particle size distributions for wheats grown at different localities were not affected similarly by different sieve sizes.

From the results obtained in this investigation it would appear that particle size distribution is a more sensitive measure of variations in flour milling quality than flour yield, and probably flour ash content.

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EFFECT OF SOY FLOUR ON AMYLOGRAMS[1]

C. W. Offelt, A. K. Smith, and James M. Mills[2]

ABSTRACT

Studies with the amylograph demonstrated that 5% of added raw soy flour has a liquefying action on starch pastes nearly equivalent to that produced by 15 S.K.B. units of malt alpha-amylase; this effect was not caused by malt beta-amylase. The factor responsible for liquefaction of starch is heat-labile, and is inactivated by raising the pH to 11.0 or lowering the pH to 2.0.

Commercial soy flours have received sufficient heat treatment to inactivate the factor responsible for liquefaction of starch pastes.

Orestano (4) reports that the soybean contains only one amylase, probably beta-amylase. Teller's studies (6) indicate the presence of two amylases in soybeans. Laufer, Tauber, and Davis (2), using the methods developed by Sandstedt, Kneen, and Blish (5) and Kneen and Sandstedt (1) for measuring the amylase activities of malt, confirm the findings of other workers that soybeans contain very little, if any, alpha-amylase. They also determined that germination does not increase either the alpha-amylase or the dextrinogenic activity of soybeans. Newton and Naylor (3), in discussing their work on soybean amylase, state: "The work in this laboratory lends support to its characterization as beta-amylase, with only a possible slight trace of alpha-amylase, both before and after germination. The only evidence that points toward the presence of alpha-amylase in soybean amylase is the marked ability of the concentrate to reduce the viscosity of starch paste."

Repeated observations in this laboratory on the increased mobility of doughs containing soy flour, and the importance of dough-handling properties to the commercial baker, prompted an examination of the effects of soy flour and of modified soy flour on amylograms.

This paper presents results obtained upon the addition of raw soy flour, commercial defatted soy flour, and soy flours modified by heating and altering their pH values on the viscosity of starch as measured by the amylograph. The effects of the various soy flours on starch viscosities are compared with viscosity effects of malt alpha-amylase and malt beta-amylase on the same starch.

Materials and Methods

The wheat starch was prepared from a hard winter wheat in the pilot plant at the Northern Utilization Research Branch. The raw

[1] Manuscript received June 7, 1954. Contribution from the Northern Utilization Research Branch, Peoria, Illinois, one of the Branches of the Agricultural Research Service, U.S. Department of Agriculture. Presented at the Annual Meeting, Denver, Colorado, May 24-27, 1954.

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defatted soy flour was prepared in the laboratory from a composite of several soybean varieties. The beans were cracked into grits between corrugated rolls, the hulls removed by aspiration, and the grits tempered and flaked between smooth rolls. The oil was removed from the flakes by extraction with hexane. The flakes were desolventized by aeration and ground in a hammer mill. The commercial defatted soy flours were taken from samples which had been obtained during a current survey of the production of the four major producers of soy flour. The soy flours subjected to steam treatment were obtained by exposing raw soy flour to atmospheric steam in an autoclave for periods of 5 to 10 minutes, respectively, and allowed to cool quickly at room temperature.

The alpha- and beta-amylases used were purified preparations from malt and were obtained from Kurth Malting Company in Milwaukee, Wisconsin.

The procedure used in this study for obtaining each amylogram is as follows: The suspension consisted of 50 g. of wheat starch, 2.5 g. of the soy sample under test, 45 ml. of pH 5.3 buffer solution, and 405 ml. water. The buffer solution was made by dissolving 26.8 g. crystalline disodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) and 10.5 g. citric acid in water, and diluting to 1000 ml. The solids were suspended in the diluted buffer solution in such a way that a minimum of air was incorporated, the suspension placed in the amylograph bowl at 30°C., and the amylograph started with the controls set to provide a temperature rise of 1.5°C. per minute. The temperature rise was arrested at 92°C., and the suspension left in the amylograph for a suitable period until the curve had passed its maximum height.

Results and Discussion

A series of amylograms designed to show the effect of raw soy flour on the viscosity of starch paste in relation to the effects of purified

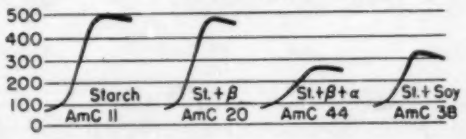

Fig. 1. Amylograms showing the effect of amylases and raw soy flour on gelatinization of starch. Curve AmC 11, starch alone; AmC 20, starch with an excess of β-amylase; AmC 44, starch plus excess β-amylase plus α-amylase; and AmC 38, starch with 5% of defatted raw soy flour.

alpha- and beta-amylases is shown in Fig. 1. The change in viscosity of a starch suspension on heating is shown in curve AmC 11. Curve

AmC 20 shows that this viscosity is not affected by the addition of 78 S.K.B. units (an excess) of purified malt beta-amylase. The liquefying action of alpha-amylase is shown in curve AmC 44 where 15 S.K.B. units of purified malt alpha-amylase have been added in addition to the 78 units of beta-amylase used in the previous test. The effect of addition of 5% of defatted raw soy flour on the viscosity of a starch paste is shown in curve AmC 38. These curves confirm that beta-amylase has a very small effect on the viscosity of starch paste, whereas alpha-amylase has a marked effect. They further show that raw soy flour contains an amylolytic enzyme which has an action on starch paste viscosity similar to that from a combination of alpha- and beta-amylases in which the viscosity change is attributed primarily to the alpha-amylase. The change in viscosity produced by addition of 5% of raw soy flour is estimated to be equivalent to the effect obtained by addition of 12 S.K.B. units of alpha-amylase.

The effect of heat-treatment of soy flour on its capacity to reduce the viscosity of starch pastes is shown by the curves in Fig. 2. The

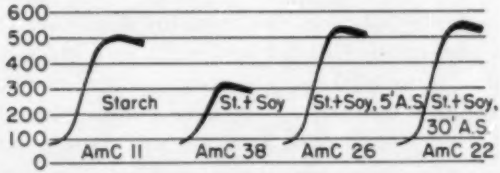

Fig. 2. Amylograms showing the result of heating raw soy flour on its effect on starch paste viscosity. AmC 11, starch alone; AmC 38, starch with 5% of raw soy flour; AmC 26, starch with 5% of soy flour steamed 5 minutes; and AmC 22, starch with 5% of soy flour steamed for 30 minutes.

flakes, from which the soy flour used in curve AmC 26 was made, had been heated in atmospheric steam for 5 minutes. The flakes used to prepare the soy flour used in curve AmC 22 had been heated in atmospheric steam for 30 minutes. These curves show that the factor in raw soy flour which causes a reduction in viscosity in starch paste is heat-labile and is readily destroyed by a very light treatment with moist heat.

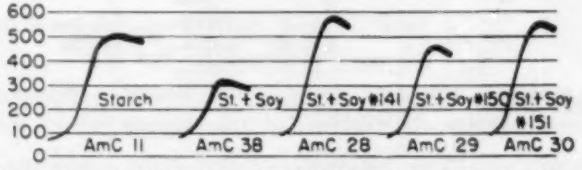

Fig. 3. Comparison of effects of commercial defatted soy flour with the effect of raw soy flour on amylograms. Curve AmC 11, starch alone; AmC 38, starch plus 5% of raw soy flour; and Curves AmC 28, AmC 29, and AmC 30, starch plus 5% of commercial defatted soy flour.

The amylograph curves in Fig. 3 show a comparison of the starch paste-liquefying effects of three commercial soy flours in comparison with that of a raw soy flour. Two of the commercial soy flours, No. 141 and No. 151, are representative of a majority of commercial soy flours and show no amylolytic activity. Therefore, the heat-treatment normally applied to soy flours in commercial processing is sufficient to destroy the factor responsible for liquefying starch pastes. The curve AmC 29 shows that soy flour No. 150 had not received sufficient heat-treatment to destroy the starch-liquefying factor. This soy flour was also the only one, out of 20 samples obtained in a survey of commercial soy flours, which caused a pronounced bleaching action when it was added to a bread dough, indicating that the lipoxidase in this sample had not been destroyed.

The effect of changing pH on the factor responsible for liquefaction of starch pastes was studied by slurrying the raw flour in water at room temperature, increasing or decreasing the pH with sodium hydroxide or hydrochloric acid, and immediately neutralizing back to the original pH before using the slurry in the amylograph. Some of these slurries were dried by lyophilization and the material was used in the dry form.

In order that the effects observed through change of pH and neutralization should not be ascribed to a salt effect, the amounts of acid and base used to obtain a definite effect were mixed together and added as a salt solution to the soy prior to use in the amylograph. Curves obtained with the described pH changes are shown in Figs. 4, 5, and 6.

Curve AmC 69, Fig. 4, shows that the starch-liquefying factor in soy

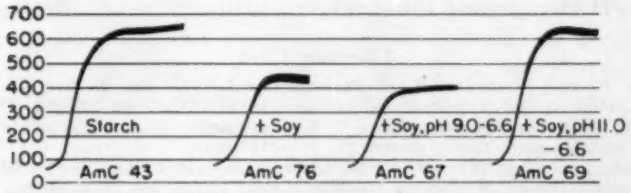

Fig. 4. Amylograms showing how pH changes in the alkaline range modify the activity of amylases in raw soy flour. Curve AmC 43, starch alone; AmC 76, starch plus 5% of raw soy flour; AmC 67, starch with 5% of raw soy flour which has been changed to pH 9.0 and back to 6.6; and AmC 69, starch plus 5% of soy flour which has been changed to pH 11.0 and back to 6.6.

flour is destroyed by exposure to a pH of 11.0, with no return of activity upon readjustment to the original pH of 6.6. Curve AmC 67, also in Fig. 4, demonstrates that the liquefying factor is stable at pH 9.0. Curves AmC 64 and AmC 62, Fig. 5, show that the starch-liquefy-

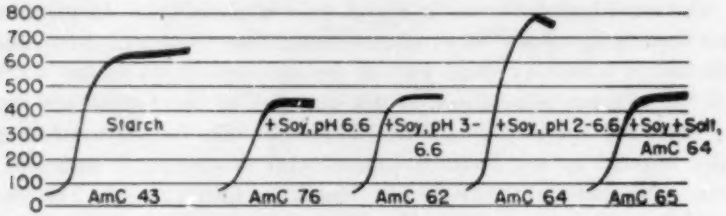

Fig. 5. Amylograms showing how pH changes in the acid range modify the activity of amylases in raw soy flour. Curve AmC 43, starch alone; AmC 76, starch plus 5% of raw soy flour; AmC 62, starch plus 5% of raw soy flour reduced to pH 3.0 and neutralized back to pH 6.6; AmC 64, starch plus 5% of defatted soy flour reduced to pH 2.0 and neutralized back to pH 6.6; AmC 65, starch plus 5% of soy flour plus the salt equivalent used in AmC 64.

ing factor is stable at pH 3.0 but is labile at pH 2.0. The curves in Fig. 6 demonstrate that lyophilization of the material had no effect on

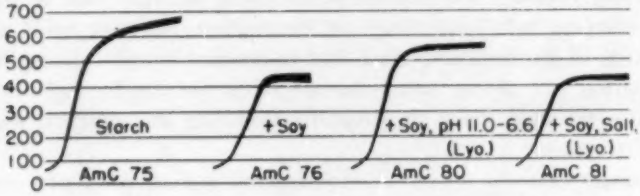

Fig. 6. Amylograms showing that neither lyophilization nor addition of the salt equivalent of the acid and base used in Figs. 4 and 5 is responsible for the observed effect. AmC 75, starch alone; AmC 76, starch plus 5% of raw soy flour; AmC 80, starch plus 5% of soy flour (adjusted to pH 11.0 in a slurry, neutralized back to pH 6.6, and dried by lyophilization); AmC 81, starch with 5% of raw soy flour plus the salt equivalent used in curve AmC 80 (lyophilized before use).

the starch-liquefying properties of soy flour, and curves AmC 65, Fig. 5, and AmC 81, Fig. 6, show that the effects previously observed were due to pH changes and could not be attributed to salt effects.

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PROTEASES OF THE SOYBEAN[1]

C. W. OFELT, A. K. SMITH, AND JAMES M. MILLS[2]

ABSTRACT

Proteases of soy flour are not water-dispersible with usual extraction technics. Methods for their determination in aqueous systems have previously been unsuccessful. The proteases of soy flour are associated with a water-insoluble component from which they can be made water-extractable by treatment of a suspension with sonic waves or through extremely rigorous mechanical treatment such as that with the Waring Blendor.[3] By use of the latter method of extraction, in conjunction with a modified Ayre-Anderson technic, a new method of assay was developed.

Raw soy flour contained three to four times the amount of protease as wheat flour, but the protease values for properly heat-treated soy flours were so low that they should have little or no effect on bread dough properties.

Investigations on the baking behavior of soy flour in bread in this laboratory have confirmed the fact that raw (nonheat-treated) soy flour has a very marked softening action on dough. The steam treatment used in producing soy flour so reduces the softening effect that it does not interfere with normal bakery operations when optimum oxidation is used. Since the softening component in most soy flours is not completely destroyed, it has been necessary to mix doughs containing soy flour stiff at the mixer in order to obtain optimum dough consistency at the time of panning. Also, in semicommercial baking tests, the last pieces passing through the molder from a large dough were noticeably softer than the first pieces molded from the same dough.

In studies of soy flour as a bread ingredient, Bohn and Favor (2) used the farinograph, in a manner described by Ford and Maiden (4), to determine the gluten-softening or proteolytic effect. They state that "soy flour causes no softening of the gluten during fermentation such as is experienced when papain is present," and conclude that the action of soy flour is not due to a protease-activating effect.

Bohn and Favor (2) also compared the action of soy flour with that of glutathione and stated that doughs with glutathione produced the same type of loaf as doughs with soy flour additions. They demonstrated that the observed effects could be completely overcome by the

[1] Manuscript received June 7, 1954. Contribution from the Northern Utilization Research Branch, Peoria, Illinois, one of the Branches of the Agricultural Research Service, U. S. Department of Agriculture. Presented at the Annual Meeting, Denver, Colorado, May 23-27, 1954.

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[3] The mention of any firm name or commercial products by trade names does not constitute an endorsement of them by the U.S. Department of Agriculture.

use of oxidizing agents and that under conditions of optimum oxidation bread containing soy flour was comparable in quality to bread made without soy flour.

Finney (3) demonstrated that excellent bread can be made using blends of soy flour with hard red spring wheat which contained up to 8% soy flour, if optimum oxidation is used. Ofelt *et al.* (14, 15) have surveyed the oxidation requirements of a number of commercial soy flours when used with an average-to-weak southwestern hard red winter wheat flour. With 5% of added soy flour, the potassium bromate requirements, based on the wheat flour, were 1.0 to 3.5 mg. % for full-fat soy flours and 3.0 to 4.0 mg. % for defatted soy flours.

Read and Haas (16) studied the effect of a water extract of soybean flour on the action of papain, bromelin, takadiastase, and trypsin on gelatin. Trypsin and the protease in takadiastase, but not papain and bromelin, were inhibited by the soy flour extract. Johnsen and Smith (5), in their studies on the hydrolytic treatment of soybean protein with papain, concluded that soybean meal contains a papain activator, suggesting the presence of a reducing component in raw soybean meal. Learmonth (7, 8, 9) concludes, from studies on the rate of liquefaction of gelatin with papain at pH 5.0, that soybean meal contains a papain inhibitor. However, unpublished results by the authors, from experiments similar to Learmonth's gelatin liquefying tests but at pH values between 7.0 and 8.0, showed a papain-activating rather than an inhibiting effect, indicating that Learmonth's interpretation of an inhibiting effect may be questioned. Therefore, it is not certain whether the dough-softening action of soy flour is due to enzyme action on the gluten fraction of the flour or to some other mechanism.

Laufer, Tauber, and Davis (6) studied the proteases of soybeans by essentially the method of Willstätter *et al.* (17) as modified by Linderström-Lang and Sato (11), and proposed the name "Soyin" for the protease system of the soybean. The use of the name "Soyin" for the proteases should not be confused with the same term "soyin" introduced at a later date by Liener (10) to describe a toxic protein in soybeans. Laufer *et al.* found that the proteases were not extractable with water but were extracted with 30% and 50% glycerol solutions. The activity of "Soyin" on casein and gelatin substrates increases rapidly with increasing pH, until an optimum is reached at approximately pH 6.0–7.0, and decreases slightly in the alkaline range to pH 9.0.

Because of the importance of, and increased interest in, protease systems in baking and lack of information in the literature on the proteases of the soybean, the present study was initiated to determine the amount and type of protease present. This paper will deal with

methods used for determination of protease, development of an improved method, and assay of results obtained on commercial-defatted soy flours available to the baking industry as well as those obtained on raw, or unheated, soy flours.

A Method for Assaying the Proteolytic Activity of Soybean Products

The method employed by Laufer *et al.* (6) yielded results which were considered unsatisfactory since the end-point was difficult to ascertain. Duplication of results was not easily obtained by a single operator and deviations between operators were even greater. Also, it was believed that the enzyme system should be studied under conditions more comparable to those found in bread doughs rather than under those existing in 30% and 50% glycerol extracts. With this in mind, an attempt was made to measure the amount of protease in a raw soy flour by using the Ayre-Anderson method as modified by Miller (12). Preliminary results confirmed the observation of Laufer *et al.* (6) that there was little or no activity in a simple water extract (pH 6.6) of soy flour. Definite activity was obtained from an extract of soy flour made with a 50% aqueous-glycerol solution at a 10:1 ratio of solvent to flour. A similar extract was made using 1 *N* sodium chloride solution as solvent. Although some activity was obtained with the latter, it was less than half that obtained with the glycerol extract.

In a further attempt to extract protease without the use of glycerol, 6 g. of soy flour were dispersed in 60 ml. water and subjected to sonic vibration by a 750-watt Raytheon 10-kilocycle oscillator with magnetic transducer, for 1 hour. This slurry was then centrifuged at 16,000 × gravity for 10 minutes in a Servall angle centrifuge and enzyme activity determined in the supernatant liquor. This treatment yielded an extract with an activity greater than that obtained in the glycerol extract.

Since supersonic generators are not generally available, and are expensive, and since the preparation of sample material by this means is exceedingly slow, it was reasoned that other mechanical methods of activating or freeing the enzyme might be as effective and more rapid. Preliminary trials showed that it was possible to get a maximum activity, equal to that obtained in a glycerol extract, by mixing 6 minutes in a Waring Blendor if the mixture were kept cool and foaming were inhibited by use of a specially designed stopper which eliminated free air space above the liquid.

Results of the above comparisons of enzyme activity obtained by different extraction technics and measured in a neutral pH range by

the Ayre-Anderson method, expressed as mg. nitrogen/10 g. of raw soy flour, are given in Table I. The results with extracts from the soy flour in the Waring Blendor are equal to those with 50% glycerol solution

TABLE I

THE EFFECT OF METHOD OF EXTRACTION ON APPARENT PROTEASE ACTIVITY AS DETERMINED BY THE MODIFIED AYRE-ANDERSON METHOD

Method of Extraction	Enzyme Activity
	mg. N/10 g. soy flour
Shaking, water–flour, 10:1	None
Shaking, 1 *N* NaCl–soy flour, 10:1	8.1
Shaking, 50% glycerol–soy flour 10:1	19.8
One-hour disintegration in sonic device, water–soy flour, 10:1	25.0
Six minutes in a Waring Blendor (chilled with ice), water–soy flour, 10:1	20.2
One-hour disintegration in sonic device, 1 *N* NaCl–soy flour, 10:1	8.1

and, although not quite as high as those with ultrasonic treatment, they are obtained far more rapidly and simply. Sodium chloride apparently partly inhibits proteolytic activity.

TABLE II

THE EFFECT OF "ACTIVATORS" ON THE PROTEASE ACTIVITY OF AQUEOUS EXTRACTS OF RAW SOY FLOUR DETERMINED BY THE MODIFIED AYRE-ANDERSON METHOD

Method of Extraction, Material Extracted, and Extracting Solvent	Enzyme Activity
	mg. N/10 g. soy flour
6 Min. in Waring Blendor, soy + 0.05% $KBrO_3$–water, 1:10	23.6
6 Min. in Waring Blendor, soy + 1.67% NaCN–water, 1:10	19.8
6 Min. in Waring Blendor, soy + 7.68% cysteine · HCl–water, 1:10[a]	1.8
6 Min. in Waring Blendor, soy + 7.68% neutralized cysteine · HCl–citrate buffer, pH 4.7, 1:10	18.6
6 Min. in Waring Blendor, soy–water, 1:10	21.8
Malted wheat flour–water, 1:4	5.0
Malted wheat flour 10, soy flour 2–water 40	12.4

[a] At a low but undetermined pH value.

Effect of Reducing and Oxidizing Agents on Activity. The effects of several reducing compounds and of potassium bromate on the activity of the protease were determined on extracts of soy flour prepared in the Waring Blendor. The tests included 0.05% potassium bromate, 1.67% sodium cyanide, and 7.68% cysteine-hydrochloride (based on the soy flours). Similar extracts were made from 1) soy flour with the cysteine-hydrochloride neutralized with sodium hydroxide and buffered to pH 4.7 with citrate buffer; 2) soy flour plus cysteine-hydrochloride in citrate buffer, pH 4.7; 3) malted wheat flour in water; and 4) malted wheat flour plus soy flour (10 wheat flour and 2 soy flour). Values for protease activity for these extracts are given in Table II. None of the oxidizing or reducing agents either activated or inactivated the protease system of soy flour.

The Ayre-Anderson Method. A single soy extract was assayed by the modified Ayre-Anderson method at various pH values to determine the optimum pH level. This method gave values for "enzyme activity" which increased steadily with a decrease in pH to 0.5. Typical values obtained are as follows:

Effect of Varying pH Values for Enzyme Activity as Determined by the Modified Ayre-Anderson Method Using Bacto Hemoglobin as Auxiliary Substrate

pH	Protease Activity expressed as mg N/10 g soy flour
1.0	65.0
3.0	46.8
5.0	28.2
7.0	20.6

This procedure must be measuring a reaction other than hydrolysis by the protease, but no explanation can be given for this anomalous behavior.

These results raised the question whether proteolytic activity was truly present in the soy extract. The presence of a protease was verified by application of the milk-clotting procedure employed by Balls and Hoover (1), and comparison was made with other enzymes to obtain a qualitative evaluation of the relative activity of the soy protease. Results of these experiments are tabulated in Table III. They show that the protease activity in raw soy flour was of the same order of magnitude as that of the malted wheat flour used for comparison.

Selection of a Substrate and Method. Wheat gluten, gelatin, and casein were tried as substrates in an effort to find a system which would behave in a rational manner with varying pH. Gluten proved

TABLE III

MILK-CLOTTING TIMES OF SEVERAL ENZYMES AS DETERMINED BY THE METHOD OF BALLS AND HOOVER

Source of Enzyme	Dispersing Agent	Ratio of Dispersing Agent to Enzyme Source	Coagulation Time
Crude papain	water	40:1	8 minutes
Crude papain	water	80:1	17 minutes
Raw soy flour	water	10:1	4.5 hours
Malted Wheat Flour	water	1.25:1	1 hour
Patent wheat flour	water	1.25:1	>20 hours
Raw soy flour	water	3:1	2.5 hours
Raw soy flour	50% glycerol	3:1	1.75 hours

to be unsatisfactory under existing conditions, since there was little development of nonprotein nitrogen upon digestion. With gelatin, it was necessary to use such a large amount of trichloroacetic acid (TCA) as a precipitant that a normality of greater than 1.3 of the acid was obtained. Soy protein is peptized in solutions of TCA at concentrations slightly higher than 1.3 *N*, and it was not deemed advisable to consider gelatin further.

The use of casein as an auxiliary substrate proved to be quite satisfactory and yielded values considerably higher than those obtained with Bacto Hemoglobin, a desirable factor where the effects of treatment to reduce activity are to be studied. The casein used was vitamin-assay grade obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and good agreement was obtained with different lots of casein. The casein dispersion was prepared by adding 20 g. to 150 ml. water, adding sufficient sodium hydroxide to bring to pH 7.5, and making up to 200 ml.

For digestion, each tube contained 10 ml. of casein dispersion, 15 ml. of phosphate-citrate buffer, and 10 ml. of enzyme extract. Five milliliters of TCA (1.43 g/ml) were used for precipitation. This value of TCA was determined, by experiment, to yield maximum protein precipitation.

With the above quantities, a 5-hour differential digestion period at 40°C., and a zero time for the blank, a pH curve was determined using appropriate buffer values and water extracts of raw soy flour. The extracts were prepared by mixing 20 g. of soy with 200 ml. of water for 6 minutes in the Waring Blendor and centrifuging for 10 minutes at top speed in the Servall angle centrifuge (approximately 16,000 × g.). Twenty milliliters of the filtrate obtained after the pre-

cipitation with TCA were used for determination of nonprotein nitrogen by the Kjeldahl method.

The effect of pH upon enzyme activity is shown in the table below, with high activity in the pH range of 4.0 to 6.0 and optimum activity at about pH 5.0.

Effect of Varying pH on the Protease Activity of Water Extracts of Raw Soy Flour Using Casein as an Auxiliary Substrate in the Modified Ayre-Anderson Method

pH	Activity expressed as mg N/10g soy, 40°C.
1.0	16.8
2.0	45.2
3.0	25.8
4.0	66.0
5.0	68.6
6.0	60.6
7.0	16.6

The enzyme activity of a raw soy flour was determined eleven times over a period of 5 days and the results were analyzed statistically. An average value of 71.2 was found with a standard error of 3.08 for a single determination.

Possible synergism between the proteases of soy and wheat flours was looked for by determining the protease activity of soy extract, wheat flour extract, and the two together. These values were determined at pH 4.7, which Miller (12) found to be best for wheat flour. Results are shown in the following table:

Protease Activity of Extracts of Soy and Wheat Flours and Mixtures of the Two, Determined by the Modified Ayre-Anderson Method with Casein as Auxiliary Substrate

Source of extract	Protease activity expressed as mg N/10 g substrate/5-hour digestion at 40° C.
Raw soy flour	69.0
Patent wheat flour	20.0
Raw soy flour + patent wheat flour	90.4

The value for wheat flour corresponds to values given by Miller (12) for patent wheat flour using Bacto Hemoglobin as an auxiliary substrate. There is no evidence of synergism since the value obtained for the combination of wheat and soy is equivalent to the total of the separate determinations.

Survey of the Proteolytic Activity of Soybean Products

The method described above was used to survey the protease activity of commercial defatted soy flours obtained from four major

producers over a 5-month period and the results are given in Table IV. Only one of the twenty samples examined had a protease activity equal to that of ordinary patent wheat flour. Thus, the deleterious

TABLE IV

PROTEASE ACTIVITY OF AQUEOUS EXTRACTS OF COMMERCIAL DEFATTED SOY FLOURS OBTAINED FROM FOUR MAJOR PRODUCERS OVER A FIVE-MONTH PERIOD AND DETERMINED BY THE MODIFIED AYRE-ANDERSON METHOD USING CASEIN AS AUXILIARY SUBSTRATE

Mill No. 1		Mill No. 2		Mill No. 3		Mill No. 4	
Sample No.	Activity[a]	Sample No.	Activity[a]	Sample No.	Activity[a]	Sample No.	Activity[a]
130	2.4	131	6.4	132	1.8	133	6.9
137	2.1	136	1.1	134	3.6	135	4.8
141	5.6	139	3.2	138	5.8	140	9.1
150	18.3	149	4.0	153	1.0	144	4.4
157	4.4	152	4.2	154	2.8	151	3.7

[a] Expressed as mg N/10 g soy for 5-hour digestion at 40°C.

effect of some soy flours on bread characteristics is probably due to some factor other than protease activity. As a matter of interest, sample No. 150, the only soy flour which had a protease activity equal to that of patent flour, was the one which had a definite bleaching effect on bread crumb, showing that this soy flour may have contained active lipoxidase.

In an effort to simplify the determination of protease, samples were assayed using a slurry of the soy flour instead of the water extract. The slurry gave higher values than the water extract; however, the pH of optimum response (about 5.5) was approximately the same as that determined on the extract. Values obtained at the various pH levels with two different buffer systems on the soy flour slurry are given in Fig. 1. The optimum pH lies between 5.25 and 6.0, and there is little difference between citrate- and acetate-buffer systems.

Discussion

Although the preceding work has not resolved questions as to the cause of the deleterious action of raw soy flour in bread doughs, it has provided a further insight into the character and amounts of protease present in soy flours.

The milk-clotting action of the water extracts of soy flour verifies previous indications that there are proteases of the papain-type present in soy flour. These proteases appear to be bound to some water-

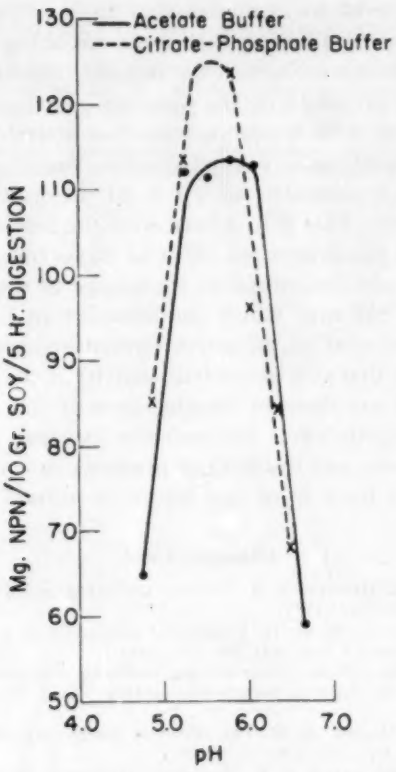

Fig. 1. Relationship between pH and protease activity in unheated soy flour with acetate- and citrate-type buffers.

insoluble component, because they are not extractable with water except after extremely rigorous mechanical treatment such as sonic energy at a high level or beating in a Waring Blendor. Even such treatment is not wholly effective, since simple slurries of the soy flour showed an appreciably higher activity than could be obtained in an extract prepared by any other available method. The association of the protease with a water-insoluble component may explain the lack of information in the literature on the proteases of soybeans, since most methods have been based upon aqueous extraction.

The very satisfactory results obtained through the use of vitamin assay-grade casein as an auxiliary substrate and the difficulties encountered in the use of Bacto Hemoglobin and wheat gluten indicate the care that must be employed in selecting a procedure for the evaluation of proteases in unfamiliar systems.

Experimental evidence indicates that the activity of proteases of soy flour were not modified by either the oxidizing or reducing substances tested. There was, however, a definite indication that the protease activity was decreased by the presence of sodium chloride in the extraction medium. This would indicate that activity in bread doughs would be minimized, since normal doughs contain sufficient sodium chloride to yield a normality of 0.5 if all the water used were free water in the system. This is in accord with the findings of Miller and Johnson (13) that the deleterious effect of excess fungal protease could be minimized by addition of salt to the sponge in bread baking.

The range of pH over which the proteases of soy flour are most active falls within that of an active fermenting bread dough. It is. however, unlikely that this proteolytic activity is the cause of deleterious properties of soy flour in doughs, since it has been shown that there is no synergistic effect between the proteases of soy flour and those of wheat flour, and the level of proteases in commercial-defatted soy flours is but a fraction of that found in normal wheat flour.

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SOURCES OF ERROR IN MICROBIOLOGICAL DETERMINATIONS OF AMINO ACIDS ON ACID HYDROLYSATES.

II. APPARENT LOSS OF AMINO ACIDS ON STORAGE[1]

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ABSTRACT

If acid hydrolysates of raw and cooked barley are not filtered properly and assayed at once, certain amino acids tend to disappear from the hydrolysates after storage at pH 6.8 under toluene at room temperature. Rates of loss vary with different amino acids, depending on the food. This loss is apparently not due to bacterial decomposition. Filtration at pH 4.0 removes the material responsible for these losses. With this technic, satisfactory recoveries of amino acids have been made from carbohydrate-containing foods, acid-hydrolyzed separately and together.

In a previous paper (5) it was shown that the humin from acid hydrolysates of certain foods contains something that interferes with the microbiological determination of correct amino acid values. It was also observed that if an acid hydrolysate is not prepared properly, and microbiological assays of its amino acid content are not made within a limited time, there is an apparent loss of amino acids. Changes take place quite rapidly in some hydrolysates and the amino acids affected vary with different foods. When a carbohydrate-containing food is hydrolyzed with acid, losses of amino acids are sometimes charged to destruction (4).

While routine microbiological assays for nine of the essential amino acids in acid hydrolysates of legumes and cereal grains were being made in this laboratory, it was observed that the values for certain amino acids such as methionine, arginine, and histidine after storage of the hydrolysate frequently were low when compared with values obtained previous to storage. Several times it was found that arginine and methionine in the hydrolysates of some foods had disappeared entirely. For example, an acid hydrolysate of barley assayed immediately after hydrolysis gave 6.00% arginine and 1.49% methionine, whereas the same solution after storage at pH 6.8 for 2 months gave 3.00% arginine and 0.50% methionine. Similarly, an acid hy-

[1] Manuscript received February 23, 1954. This is the second paper dealing with sources of error on amino acid assays of acid hydrolysates of certain foods from Human Nutrition Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Washington 25, D.C.

drolysate of black-eyed peas assayed at once gave 8.0% arginine, but after storage for 1 month gave a zero value for arginine. A hydrolysate of brown rice stored for 3 weeks showed no arginine, while a hydrolysate of white rice after the same storage period assayed 8.39% arginine. A hydrolysate of kidney beans assayed at once gave 2.91% histidine, but after standing for 3 weeks assayed 0.74% histidine. These observations led to an investigation of the cause for such losses and to an explanation of some of the reported destruction of amino acids when proteins are hydrolyzed in the presence of carbohydrate.

Since it is well known that cooking or processing modifies food proteins in some way (1, 2, 3), it was thought possible that cooked foods might show some destruction of amino acids when they were hydrolyzed with acid. Therefore, in the present study assays were made of amino acids in both raw and cooked barley.

Materials and Methods

An experiment was designed to illustrate the effect of cooking and also the effect of various methods of filtering on the loss of amino acids during storage of the hydrolysates.

Five hydrolysates of barley (1 g. barley each) were prepared in the following manner: 1) raw barley refluxed for 24 hours with 35 ml. of 20% hydrochloric acid, adjusted to pH 6.8 and diluted to volume without filtering; 2) raw barley refluxed for 24 hours with 35 ml. of 20% hydrochloric acid, heated to boiling with norite (0.5 g.), filtered, adjusted to pH 6.8, and diluted to volume; 3) barley cooked by autoclaving 45 minutes at 15 lb. pressure in the presence of water, refluxed for 24 hours with 35 ml. of 20% hydrochloric acid, adjusted to pH 6.8, and diluted to volume without filtering; 4) barley cooked by autoclaving 45 minutes at 15 lb. pressure in the presence of water, refluxed for 24 hours with 35 ml. of 20% hydrochloric acid, heated to boiling with norite (0.5 g.), filtered, adjusted to pH 6.8, and diluted to volume; and 5) raw barley refluxed for 24 hours with 35 ml. of 20% hydrochloric acid, adjusted to pH 4.0,[2] filtered through a fritted glass funnel (Pyrex M), adjusted to pH 6.8, and made up to volume.

Using the procedures of Horn *et al.* (6), the solution filtered at pH 4.0 was assayed microbiologically for nine amino acids: at once, after 2 weeks, and after 1 month; the remaining four solutions were assayed at once, after 3 weeks, and after 2 months. The solutions were stored under toluene at room temperature.

[2] It has been found in this laboratory that pH 4.0 was the best hydrogen-ion concentration for removing the maximum amount of humin from hydrolysates.

Results and Discussion

Table I shows that rapid loss of arginine, histidine, and methionine occurred in the unfiltered hydrolysate of raw barley. After 3 weeks arginine, histidine, and methionine values were lower than when assayed at once; after 2 months, not only arginine, histidine, and methionine, but also lysine and valine values were low compared with the value when assayed at once, methionine having almost completely disappeared. As pointed out in our first paper (5), the values for some

TABLE I

EFFECT OF TREATMENT AND STORAGE OF HYDROLYSATES ON THE VALUES OF NINE AMINO ACIDS OF RAW BARLEY[a]

Amino Acid	RAW–UNFILTERED			RAW–CARBON-FILTERED			RAW–FILTERED pH 4.0			Average Values, Columns
	When Assayed			When Assayed			When Assayed			
	At Once	3 Weeks	2 Months	At Once	3 Weeks	2 Months	At Once	2 Weeks	1 Month	
	1	2	3	4	5	6	7	8	9	4, 7
Arginine	5.82	5.33	3.64	4.77	4.69	4.77	4.61	4.74	4.52	4.69
Histidine	2.18	1.68	1.02	2.10	2.01	1.62	2.10	2.12	2.07	2.10
Isoleucine	4.36	4.20	4.10	4.04	4.20	4.20	4.05	4.09	4.09	4.05
Leucine	6.54	6.38	6.59	6.63	6.38	6.55	6.82	6.85	6.85	6.73
Lysine	4.28	4.08	3.47	3.64	3.64	3.64	3.68	3.64	3.72	3.66
Methionine	1.49	0.97	0.09	1.44	1.36	1.35	1.38	1.39	1.33	1.41
Phenylalanine	5.20	5.09	5.14	3.51	3.29	3.26	5.20	5.33	5.17	5.20[b]
Threonine	3.40	3.56	3.47	3.56	3.47	40	3.50	3.31	3.47	3.53
Valine	6.79	6.79	5.90	5.21	5.25	5.41	5.30	5.25	5.33	5.26

[a] Calculated to 16% nitrogen.
[b] Average, columns 1, 7.

of the amino acids when assayed at once are high owing to the effect of the humin, and it was at first thought that the losses of amino acids on storage might be due to loss of the activity of the humin, but the losses of arginine, histidine, and methionine go far beyond the effect attributable to the humin.

The carbon-filtered hydrolysate of the raw barley and the hydrolysate filtered at pH 4.0 did not show these rapid losses. In only one instance, that of histidine, assayed after 2 months (column 6), was any large loss shown on storage. The low values for phenylalanine on the carbon-filtered sample indicate absorption of this amino acid by carbon, and for this reason this method of filtering is not recommended. The results obtained on the hydrolysate filtered at pH 4.0 indicate that, if assayed within a reasonable length of time (one month), neither losses of amino acids nor the effect of the humin are observed.

Similar results were obtained on the hydrolysates of cooked barley and no losses of amino acids due to cooking were observed.

Table I illustrates the sources of error that can arise on amino acid assay of certain foods since the correct[3] amino acid value has been shown to be obtained from acid hydrolysates filtered at pH 4.0 (5). For example, if the hydrolysate is not filtered properly the value obtained for arginine in barley when assayed immediately is high (5.82%), due to activity of the humin (5). The value on assay after 2 months' storage is lower (3.64%) than can be accounted for by the loss of activity of the humin, since the correct value (pH 4.0 filtered) is 4.69%. This indicates an actual loss of arginine in stored unfiltered hydrolysates. The humin has no effect on values for histidine when the hydrolysate is assayed at once, 2.18% (correct value 2.10%), but losses on storage are large, after 2 months the value being 1.02%. The humin has a slight effect on isoleucine values, 4.36% (correct value 4.05%), which disappears on standing, and there is no loss on storage, value 4.10%. Humin has no effect on leucine or threonine values, 6.54% and 3.40% respectively (correct values 6.73% and 3.53%), and there is no loss due to storage, values 6.59% and 3.47%. Humin activity gives high lysine and valine values, 4.28% and 6.79% (correct values, 3.66% and 5.26%). The activity of the humin is lost on storage but there is no additional loss of these amino acids, values 3.47% and 5.90%. The humin has no effect on the methionine value, 1.49% (correct value, 1.41%), but there is a large loss on storage, value 0.09%. Humin has no effect on the phenylalanine values, 5.20% (correct value, 5.20%), nor is there any loss on storage, value 5.14%.

To show that the losses of amino acids on storage were not due to bacterial contamination, a sample of barley was hydrolyzed by reflux with 20% hydrochloric acid solution, adjusted to pH 6.8, diluted to volume without filtering, and divided into two parts. Each portion was assayed at once for methionine and histidine and they assayed 1.42% and 2.10% respectively. One portion was placed under toluene and the other was sterilized in the autoclave at 15 lb. pressure for 15 minutes and stored at room temperature for 3 months. These samples were then reassayed for the two amino acids and the part stored under toluene assayed 0.12% and 0.97% respectively, while the sterilized portion assayed 0.12% and 1.03%.

In hydrolysates of other foods such as brown rice or black-eyed peas the same factors operate but the rates of loss of amino acids are different from those found for barley.

[3] "Correct" is defined as the value obtained when all interfering factors have been compensated for (5).

TABLE II

RECOVERY OF AMINO ACIDS AT FOUR DIFFERENT ASSAY LEVELS[a] WHEN CASEIN AND BARLEY ARE HYDROLYZED SEPARATELY AND TOGETHER AND HYDROLYSATES FILTERED AT pH 4.0

Amino Acid	Amino Acid Added as Casein	Amino Acid Added as Barley	Total Amino Acid Added as Casein plus Barley	Total Amino Acid Found, Casein plus Barley	Amino Acid Recovery
	γ	γ	γ	γ	%
Arginine	3.75	6.40	10.15	9.60	94.6
	7.00	12.80	19.80	19.30	97.5
	10.60	18.50	29.10	28.80	98.9
	14.00	25.00	39.00	40.00	102.6
Average					98.4
Histidine	2.48	2.60	5.08	5.40	106.3
	5.28	5.52	10.80	10.30	95.4
	7.68	7.92	15.60	16.00	102.6
	10.30	10.70	21.00	20.65	98.3
Average					100.7
Isoleucine	5.50	5.00	10.50	10.80	102.9
	10.70	10.00	20.70	20.70	100.0
	16.20	15.00	31.20	31.40	100.6
	22.00	19.70	41.70	40.80	97.8
Average					100.3
Leucine	8.40	8.00	16.40	17.20	104.9
	17.20	16.40	33.60	34.00	101.2
	25.80	24.70	50.50	49.00	97.0
	34.60	33.00	67.60	65.00	96.2
Average					99.8
Lysine	7.40	4.50	11.90	12.60	105.9
	16.00	8.80	24.80	24.50	98.8
	24.50	14.00	38.50	37.65	97.8
	33.00	18.80	51.80	50.00	96.5
Average					99.8
Methionine	3.00	1.95	4.95	4.52	91.3
	5.40	3.80	9.20	9.20	100.0
	8.70	5.70	14.40	13.90	96.5
	11.10	7.70	18.80	18.65	99.2
Average					96.8
Phenylalanine	4.75	6.40	11.15	11.20	100.5
	9.75	12.80	22.55	20.75	92.2
	14.25	19.00	33.25	32.25	97.0
	18.75	25.20	43.95	44.20	100.6
Average					97.6
Threonine	4.00	4.00	8.00	8.20	102.5
	7.80	8.50	16.30	17.00	104.3
	11.80	13.20	25.00	24.20	96.8
	16.00	17.60	33.60	32.90	97.9
Average					100.6
Valine	6.20	6.20	12.40	12.70	102.4
	12.70	13.50	26.20	25.40	96.9
	18.70	19.40	38.10	40.80	107.1
	25.40	26.20	51.60	55.00	106.6
Average					103.3

[a] Assay level of casein, 100γ, 200γ, 300γ, 400γ; of barley, 1000γ, 2000γ, 3000γ, 4000γ.

TABLE III

AMINO ACID VALUES[a] OF VARIOUS FOOD HYDROLYSATES FILTERED AT pH 4.0 AND ASSAYED AT ONCE

Food	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine
	%	%	%	%	%	%	%	%	%
Peas, green	9.69	2.27	4.61	7.53	7.49	0.79	4.78	3.89	4.91
Beans, kidney	6.30	2.80	5.01	7.64	7.51	1.12	5.78	4.64	5.82
Beans, Navy	6.90	2.87	4.93	7.97	7.93	1.19	6.14	5.20	6.41
Beans, lima	6.38	3.03	5.59	8.18	7.58	1.24	5.64	4.76	5.78
Peas, black-eyed	7.55	3.02	4.78	8.02	7.08	1.44	5.82	4.31	5.44
Egg powder	7.02	2.45	6.48	8.89	7.90	3.26	5.40	5.24	6.73
Lentils	8.89	2.23	4.36	6.85	6.35	0.65	4.50	3.50	4.82
Rice, white	8.27	2.21	4.51	7.81	3.67	2.31	4.54	3.43	6.16
Rice, brown	8.37	2.42	4.60	7.86	3.92	2.14	4.79	3.65	6.22
Wheat, whole	4.22	2.13	4.21	6.55	2.65	1.39	4.88	2.86	4.34
Oats, whole	6.84	2.14	4.40	7.41	3.94	1.47	5.48	3.43	5.68
Corn, whole	4.23	2.92	4.12	12.68	2.42	1.96	5.21	3.74	4.89
Peanuts, Spanish	12.01	2.27	4.43	6.32	3.77	1.10	5.24	2.84	4.36
Soybean	8.22	2.60	5.51	7.50	6.75	1.27	5.00	4.15	4.74

[a] Calculated to 16% N.

When casein and whole barley were acid-hydrolyzed separately and together, filtered at pH 4.0, and assayed for nine of the essential amino acids, the sum of the amino acid values of the casein and barley when hydrolyzed separately agreed with the amino acid values found in the hydrolysate of the mixture (Table II). The presence of the carbohydrate of the barley caused no loss in the amino acid values of the casein. It is possible that the losses reported by other workers occurred after, rather than during, hydrolysis.

Table III shows amino acid values of various food hydrolysates, filtered at pH 4.0 and assayed at once.

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DETERMINATION OF THE DEGREE OF POLISHING IN RICE. I. SOME METHODS FOR COMPARISON OF THE DEGREE OF MILLING[1]

H. S. R. DESIKACHAR

ABSTRACT

In three varieties of rice, the proportion of grains with lost germs steadily increased with progressive increase in the degree of polishing. Staining of the rice grains with methylene blue followed by congo red could be used for qualitative inspection of the degree of polishing. The absorbed congo red could be eluted with pyridine.

The pigments of rice bran gave color reactions with alkaline methanol and with sodium nitrite. The intensity of color in these reactions decreased progressively on milling. Phytin values determined by a colorimetric method and an indirect estimation of fat by measuring its iodine absorption capacity showed that milling progressively reduced the phytin and fat contents in rice.

Simple and rapid procedures for measuring variations in the different characteristics of the rice grain as a result of subjection to progressive polishing have been described. Some of these procedures have been found suitable for a semiquantitative comparison of the degree of milling in rice.

For the production of undermilled rice in rice-eating countries, there is considerable need for simple and rapid procedures that can be adopted by rice millers for assessing the degree of polishing of samples of rice. Staining with iodine or Lugol's solution is being used in some countries for studying qualitatively the removal of the bran layers. Goudswaard (2) suggested the use of indigo carmine and fuchsin for staining the rice. Evaluations based on determination of thiamine content by colorimetric procedures have also been recently suggested (4, 5, 6, 7). A relationship between the fat content of the rice grain and the extent of polishing has also been reported recently by Grigorieff *et al.* (3).

Investigations are being pursued in the author's laboratory to develop rapid and simple procedures that can be used for comparative purposes in determining the extent of polishing. These procedures are based on 1) a study of the loss of germs accompanying the milling process, 2) the differential staining of the bran and endosperm, 3) measurement of residual bran pigments by suitable color tests, and 4) determination of phytin by a simplified method and indirect measurement of the fat content of the grain by its iodine absorption capacity. Major emphasis in working out these procedures has been on the rapidity with which the degree of milling of a number of samples can be compared with reasonable accuracy.

[1] Manuscript received July 14, 1954. Communication from the Division of Food Processing, Central Food Technological Research Institute, Mysore, India.

Materials

Twelve samples of rice polished to different degrees were prepared from each of three local varieties (S.749, G.E.B.24, and S.129) by passing the shelled rice through a commercial polishing cone. For measurement of the loss of germs, only whole grains were used. For other studies, the entire sample including the broken grains was pulverized to pass through a 40-mesh sieve.

Methods

Pattern of Loss of Germs. The percentage of grains with lost germs, i.e., with scooped-out germ tips (referred to here as the germ index) was determined by actual counting of lots of each sample containing 400–500 grains. Preliminary trials indicated that this number of grains randomly selected from each sample could be taken as fairly representative of the sample (variation in a typical case was 47.5 ± 2.1).

Differential Staining Characters of Bran and Endosperm. Of the various staining reagents used, congo red and methylene blue gave best differential staining to the bran and endosperm. The actual procedure used for staining was as follows: The rice grains were soaked in 0.025% methylene blue solution for 1 minute, after which the dye was decanted off and the grains washed twice with water. The blue grains were subsequently immersed in 0.5% congo red solution for 2.5–3 minutes, followed by draining and washing as before. When the grains were immersed in a small amount of water and viewed, the bran layers appeared blue while the endosperm was stained red. This method could be used for a qualitative inspection of the degree of polishing.

For quantitative work, 5 g. of the rice grains were stained with 0.5% congo red solution for 5 minutes or with 0.0125% methylene blue solution for 2 minutes. After decanting and washing as before, the adsorbed dyes were extracted by shaking with suitable solvents. In the present studies, pyridine was used to extract the congo red. A mixture of equal volumes of methanol and 0.5 *N* hydrochloric acid solution was employed to extract the adsorbed methylene blue. The comparative color intensity in the eluates was measured in a Klett-Summerson Photoelectric Colorimeter using appropriate filters (Nos. 42 and 66).

Color Tests Based on Removal of Bran Pigment. During the course of thiamine determinations in rice, it was observed that the addition of methanol and alkali to acid extracts of rice gave a red or brown color. This color was maximum with unpolished rice. Further, it was noticed that, on treatment with sodium nitrite, acid extracts of rice

gave a yellow color which turned pink when made alkaline. The intensity of this pink color decreased with progressive polishing of the rice. The following procedures were standardized for comparative work.

The alkaline methanol test consisted in shaking 5 g. of rice flour for 5 minutes with 10 ml. of distilled methanol and 10 ml. of 10% sodium carbonate solution and measuring the intensity of the brown color after filtering the contents. A blue filter (K.S. No. 42) was used for color comparison. The alkaline nitrite test was carried out by shaking 5 g. of rice flour with 25 ml. 0.5 *N* sulfuric acid for 10 minutes and adding 1 ml. of 10% sodium nitrite solution to an aliquot of the filtrate. The yellow solution was made alkaline with a known volume of 10% sodium hydroxide and filtered. The pink color of the filtrate was measured using a green filter (K.S. No. 54).

Loss of Phytin and Fat During Polishing. The following procedure based on Young's method (11) was adopted for estimation of phytin. To 5 g. of rice flour were added 30 ml. of 0.5 *N* hydrochloric acid and 20 ml. of standard ferric chloride solution (0.5 mg iron/ml). After agitation for 10 minutes it was filtered. To 1 ml. of the filtrate were added 7 ml. of 0.5 *N* hydrochloric acid and 2 ml. of 2% potassium thiocyanate solution. The red color due to excess iron was measured in a colorimeter. The amount of iron used up for precipitation by 5 g. of rice flour (referred to as the iron binding index) was calculated for the various samples of rice.

As direct estimation of micro amounts of fat is difficult, the fat was estimated indirectly by determining its iodine absorption capacity according to the method of Yasuda (10). The method consisted in shaking 5 g. rice flour with 10 ml. chloroform, filtering through a fat-free filter paper, and determining the iodine or bromine equivalent in a 2-ml. aliquot of the extract using the micro pyridine sulfate dibromide reagent of Yasuda (10).

Results

Thiamine Loss. The data in Table I show that there was a progressive loss in thiamine content as the degree of polishing was increased.

Germ Index. Table II shows that in all three varieties the germ index increases with higher degrees of milling. Although the pattern of variation is of the same type in all three varieties, the hardness of the variety of rice, type of polishing equipment used, previous conditioning and humidity of the grain, etc., affect the actual index of germ loss. The method is of value only for comparative purposes. The

TABLE I

THIAMINE IN γ/g OF RICE SAMPLES POLISHED TO DIFFERENT DEGREES
(Values are on a dry-matter basis)

Sample No.[a]	S. 749	GEB 24	S. 129
1	3.38	3.54	3.49
2	3.16	3.09	3.19
3	3.14	2.94	2.97
4	2.97	2.91	2.75
5	2.92	2.90	2.39
6	2.49	2.87	2.36
7	2.37	2.45	2.32
8	2.08	2.09	2.07
9	1.96	1.64	1.91
10	1.85	1.54	1.85
11	1.54	1.21	1.65
12	1.02	0.69	1.32

[a] The extent of polishing increases with the sample number.

simplicity of the method is, however, an advantage. Rice polished to a degree so as to have a germ index not more than 50–60 would still contain enough thiamine to prevent beri-beri.

Staining Behavior. The differential staining with methylene blue followed by congo red can be used for qualitative observations. Data presented in Table III show that while adsorption of congo red progressively increases with the degree of milling, adsorption of methylene

TABLE II

VARIATION OF GERM INDEX WITH MILLING

Sample No.[a]	S. 749	GEB 24	S. 129
1	5.8	5.6	9.2
2	12.4	16.8	18.6
3	17.8	26.7	28.1
4	25.4	25.9	37.9
5	41.6	31.1	40.4
6	40.1	39.5	41.4
7	45.9	48.4	55.9
8	73.3	64.0	73.0
9	84.1	76.7	78.0
10	90.8	82.8	78.5
11	95.0	92.0	80.4
12	97.0	94.6	92.9

[a] The extent of polishing increases with the sample number.

blue reaches a more or less steady value after a certain degree of polishing. For this reason and because the differences in the color intensity of eluates in successive samples are small, methylene blue is not a suitable reagent for studying differences in the degree of polishing. Congo red is, however, a suitable reagent for this purpose.

Color Reaction of Bran. Results in Table IV indicate that both

TABLE III

COMPARATIVE COLOR INTENSITIES OF CONGO RED AND METHYLENE BLUE ELUATES

Sample No.[a]	Congo Red (0.5% for 5 minutes)		Methylene Blue (0.0125% for 2 minutes)	
	S. 749	GEB 24	S. 749	GEB 24
1	143	121	220	198
2	153	169	193	183
3	170	240	190	170
4	163	249	185	166
5	175	261	177	165
6	190	272	163	161
7	195	281	162	162
8	220	287	161	165
9	226	300	162	160
10	267	—	158	166
11	290	367	161	161
12	305	418	161	162

[a] The extent of polishing increases with the sample number.

the alkaline methanol and nitrite tests can be used to differentiate samples of rice polished to different extents. Varietal differences in the nature and concentration of bran pigments exist, but for comparison among different samples of the same variety both tests are quite useful. The nature of the chemical reaction between the pigments and the

TABLE IV

COMPARATIVE COLOR INTENSITIES OF BRAN PIGMENTS GIVING COLOR WITH ALKALINE METHANOL AND ALKALINE NITRITE SOLUTION

Sample No.[a]	Alkaline Methanol			Alkaline Nitrite		
	S. 749	GEB 24	S. 129	S. 749	GEB 24	S. 129
1	400	420	360	220	178	128
2	350	335	335	—	—	—
3	335	282	322	165	134	—
4	328	272	312	—	—	—
5	308	262	320	143	107	106
6	294	250	305	—	—	97
7	276	242	290	120	102	—
8	263	218	282	—	—	88
9	248	206	270	107	71	82
10	226	191	261	—	—	67
11	178	182	234	57	66	—
12	178	170	222	45	55	56

[a] The extent of polishing increases with the sample number.

nitrite used above is yet obscure. It may be either a diazo type or an isonitroso type reaction.

Iron Binding Index and Iodine Absorption. Data on the iron binding index in Table V show that differences exist between successive samples, but that these differences do not become noticeable until the

TABLE V

IRON BINDING INDEX AND IODINE ABSORPTION CAPACITY

Sample No.[a]	Iron Binding Index			Iodine Absorption Capacity[b]		
	S. 749	GEB 24	S. 129	S. 749	GEB 24	S. 129
1	9.0	8.9	9.1	13.9	16.7	16.6
2	9.0	8.9	9.1	13.8	13.6	15.1
3	9.0	8.9	9.1	13.5	12.8	13.5
4	8.8	8.7	9.1	13.7	12.0	...
5	8.5	8.4	9.1	12.5	10.7	10.1
6	7.6	8.3	9.1	11.4	...	...
7	7.4	7.7	9.1	11.2	10.1	9.9
8	6.7	6.9	9.1	9.3	9.8	...
9	6.6	5.8	8.7	8.2	8.4	9.1
10	5.0	5.0	8.4	7.5	...	8.4
11	2.8	2.3	8.2	4.1	5.4	7.1
12	1.9	1.8	7.1	2.3	5.0	6.8

[a] The extent of polishing increases with the sample number.
[b] Expressed in terms of 0.01 N sodium thiosulfate solution.

rice has been polished to a certain extent. There are also differences among the three varieties. The iron binding index data do not correspond to true phytin values as reported by others (1, 8, 9). The extraction of phytin is incomplete owing to the short period of extraction. Moreover, the addition of ferric chloride during the extraction stage as adopted in the above procedure to save time hinders efficient extraction of the phytic acid by forming an insoluble iron salt with it. The procedure for extraction needs, therefore, to be modified to overcome this difficulty.

The iodine absorption capacity decreases with progressive increase in the degree of milling. A short 10-minute extraction procedure for fat as adopted here is not expected to effect complete extraction of fat. Hence the data reported above do not relate to absolute fat content in the rice. The procedure can be used, however, for comparison among samples in any one variety.

Discussion

While there is scope for refinement and improvement in the various procedures described in this paper, the suitability of such simple and rapid methods for routine and comparative purposes for use in commercial rice mills is indicated by this study. Few rice mills in India and the Far East have adequate laboratory facilities for accurate assays. Methods of the type described in this paper may therefore be of some use in such countries because of the simplicity of the methods and the rapidity with which they can be conducted.

Acknowledgment

The author is highly indebted to Dr. V. Subrahmanyan, Director, Central Food Technological Research Institute, Mysore, and Dr. D. S. Bhatia, Head of the Division of Food Processing, for their keen interest and kind encouragement during the course of this work.

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DETERMINATION OF THE DEGREE OF POLISHING IN RICE. II. DETERMINATION OF THIAMINE AND PHOSPHORUS FOR PROCESSING CONTROL[1]

H. S. R. DESIKACHAR

ABSTRACT

Shaking with dilute acid for 10 minutes extracted about 90% of the thiamine in rice flour. Similarly, digestion of rice flour with concentrated sulfuric acid even for 5 minutes released 90% of the total phosphorus.

For processing control in rice mills, the short period extraction and digestion procedures could be adopted for routine assay to effect a saving in time.

Recently Lyman *et al.* (5) suggested that in the determination of thiamine for milling control, the step of enzyme hydrolysis can be omitted to save time. As thiamine in rice is also easily leached out with water (1, 6), a reduction in the time of extraction of thiamine from rice flour was considered possible. Similarly, a reduction in time in the wet digestion method of Gerritz (3) for phosphorus determination appeared possible, as preliminary experiments showed that digestion of rice flour wth sulfuric acid even for 5 minutes gave about 90% recovery of the total phosphorus.

A shortened 10-minute extraction procedure for thiamine assays and a 5-minute digestion for phosphorus determinations were adopted to effect a substantial saving in time, and values obtained by the shortened procedures in samples of rice polished to different extents were compared with corresponding values as determined by the complete conventional procedures.

Materials and Methods

Samples of rice polished to different extents were prepared from paddy (G.E.B. 24 variety) and powdered to pass through a 40-mesh sieve. For short term thiamine extraction, 5 g. of rice flour were shaken with 50 ml. of 10 *N* sulfuric acid for 10 minutes. The mixture was adjusted to a pH of about 4 with sodium acetate solution, made up to 100 ml. and filtered through a dry filter-paper. As the thiochrome oxidation procedure could be carried out in a shorter period than the colorimetric method adopted by Lyman *et al.* (5), the former method was employed for thiamine assay. In corresponding samples, complete extraction and enzyme digestion of bound thiamine were carried out according to Kik and Williams (4).

[1] Manuscript received July 14, 1954. Communication from the Division of Food Processing, Central Food Technological Research Institute, Mysore, India.

For the shortened phosphorus digestion, 0.5 g. of rice flour was digested for 5 minutes with 2 ml. concentrated sulfuric acid in a small digestion flask over a medium flame. The contents were made up to 100 ml. and filtered. Aliquots of 5–10 ml. were used for phosphorus assay according to the colorimetric method of Fiske and Subbarow (2). A blank was run to correct for the brownish color of the solution. Complete digestion was carried out, according to Gerritz (3), and phosphorus was determined colorimetrically. A Klett-Summerson Photoelectric Colorimeter was employed for color comparison.

Results and Discussion

The thiamine and phosphorus values for the different samples as determined by the shortened as well as the complete procedures are given in Table I.

TABLE I

THIAMINE AND PHOSPHORUS CONTENTS OF RICE POLISHED TO DIFFERENT DEGREES
(Values are on a dry-matter basis)

Sample No.	Thiamine			Phosphorus		
	Total	Short-Term Procedure	Short-Term Procedure as Percent of Total	Total	Short-Term Procedure	Short-Term Procedure as Percent of Total
	γ/g rice			mg/100 g rice		
1	3.3	2.7	82	253	245	96
2	2.3	2.1	91	207	196	95
3	1.8	1.6	90	152	139	92
4	1.4	1.3	93	132	121	92
5	1.3	1.2	92	127	116	92
6	0.9	0.9	100	101	93	92

Comparison of thiamine values as determined by both methods shows that the short term method gives values about 10% lower than the total thiamine values. Lower recovery is obtained in the case of completely unpolished rice. This is perhaps due to the large amount of bound thiamine in unpolished rice which is not estimated by the shortened procedure. In eight commercial samples of rice, the recovery of thiamine by the shortened procedure ranged from 81 to 93% with a mean of 88%. If one makes allowance for the fact that the shortened procedure gives values about 10–15% lower than the true thiamine values, the 10-minute extraction procedure can be employed for routine assay in processing control. The assay can be completed in about 30 minutes.

About 90–95% of the total phosphorus can be accounted for by the short digestion procedure. For routine assay work and for com-

parative assays during milling control, the 5-minute digestion procedure can be used with advantage.

Acknowledgment

The author is indebted to Dr. V. Subrahmanyan, Director, Central Food Technological Research Institute, Mysore, and to Dr. D. S. Bhatia and Dr. M. Swaminathan of this Institute for their kind interest and encouragement during the course of this work.

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DETERMINATION OF THE DEGREE OF POLISHING IN RICE. III. THE USE OF COLORED RICE AS AN INDICATOR OF THE DEGREE OF BRAN REMOVAL IN RICE MILLING[1]

H. S. R. Desikachar

ABSTRACT

The bran pigments in a variety of red rice were extracted with hot sodium bicarbonate solution and the intensity of this color was taken as a measure of the degree of bran removal during milling. The loss of thiamine during milling was of the same order for the red rice and a white rice with which it was milled. The use of the red rice as an indicator during rice milling is suggested.

Observations during the milling of such commercial samples of paddy where white grains had naturally got mixed with a small proportion of red grains indicated that the colored grains became white on removal of the bran layers. Such varieties of rice with a colored

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pericarp and a white endosperm could be considered as grains stained by natural pigments, and it was thought that this natural property could be made use of to measure the degree of polishing in rice provided the bran color could be easily extracted and some type of correlation established between the amount of such extractable color and the thiamine content of the rice at each stage of milling. The possibility of using such colored varieties as an indicator in the milling of rice is studied here.

Materials and Methods

A commercial bulk sample of paddy (G.E.B. 24 variety) with a natural admixture of 7–8% with another variety having a colored pericarp was milled, and six samples representing increasing degrees of milling were collected.[2] The last sample had been polished to a degree just sufficient to remove the bran layers.

The pigments of the pericarp were found insoluble in organic solvents but were slowly extracted by cold water or dilute alkali (Nagai (3) and Ramiah *et al.* (4)). It was observed that the light yellow aqueous extract changed to pink when made alkaline and that boiling water extracted the color very quickly. Based on these observations, the procedure finally adopted for extracting the bran color was as follows: From a representative 5-g. sample at each stage of milling, the colored grains were removed by hand picking and transferred to test tubes containing 10 ml. of 20% sodium carbonate solution; these tubes were immersed in boiling water for 10 minutes. The contents were made up to 25 ml. and filtered. The color intensity in the filtrates was measured in a photoelectric colorimeter (Klett type) with a green filter (No. 54).

Thiamine contents in each of the samples as well as in the red and white grains separated from each of the six samples were determined by the thiochrome method using the modifications suggested by Kik and Williams (2).

Results and Discussion

The data on the intensity of bran color as well as on the thiamine contents of the six samples are presented in Table I.

The data on the thiamine contents of the various samples show that the depletion of thiamine due to milling is of the same order in

[2] **Editor's Note.** In the United States, strenuous efforts are being made to reduce the amount of red rice. According to the U. S. Grain Standards for milled rice, U. S. No. 1 cannot contain more than 0.5% of red rice and damaged kernels (other than heat-damaged), either singly or combined. Milled rice containing more than 6.0% red rice would be graded U. S. No. 6. **W.F.G.**

TABLE I

INTENSITY OF BRAN COLOR, AND THIAMINE CONTENT, OF POLISHED RICE
(Values are on a dry-matter basis)

Sample No.	Bran Color Reading	Thiamine content in γ/g rice		
		Mixed Grains	White Grains	Red Grains
1	442	3.30	3.32	3.01
2	200	2.98	3.00	2.71
3	89	2.57	2.57	2.22
4	64	2.10	2.14	1.94
5	34	1.52	1.60	1.47
6	21	1.30	1.31	1.38

both the red and white grains, indicating that their milling characteristics are about the same. The red grain can therefore be used as a suitable indicator in rice milling.

The intensity of bran color extracted by hot sodium bicarbonate solution decreases with milling and can be used for measuring the degree of bran removal during milling. The rate of loss of bran color is very rapid when compared with the rate of loss of thiamine. The bran color decreases by about 50% for a 10% loss in thiamine. For detecting very small changes in the early stages of milling, the bran color test is therefore very sensitive. From the practical point of view, the presence of the red grains helps in judging the extent of bran removed during milling. Polishing of rice to a degree corresponding to sample 4 above would remove about 85% of the bran and it would still contain enough thiamine to prevent beri-beri in rice diets (1). Such a sample would contain about 2 γ thiamine/g.

Acknowledgment

The author is highly thankful to Dr. V. Subrahmanyan, Director, Central Food Technological Research Institute and Dr. D. S. Bhatia, Head of the Division of Food Processing for their kind interest in the work.

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LABORATORY DOUGH MIXER WITH AN AIR-TIGHT BOWL[1]

I. HLYNKA AND J. A. ANDERSON

ABSTRACT

A laboratory mixer for 100 g. to 200 g. of flour is described. A closed, water-jacketed bowl makes an airtight seal with the mixer and permits mixing in any gas or under positive or negative pressure. An alternate bowl with openings permits ordinary mixing in air.

Several investigators (1, 2, 3, 5) have shown that oxygen, incorporated into dough during mixing, has a significant improver effect. For certain studies of improver action of bromate, iodate, etc., it is therefore advantageous to mix doughs in nitrogen or other inert atmosphere. Enclosing the entire mixer in a bell jar (1) and other improvisations (3) have been used but are too cumbersome for extensive investigations. Accordingly a new mixer, in which the mixing chamber can be readily evacuated and an inert atmosphere introduced, was developed. The new mixer, called the G.R.L. mixer (initials of this laboratory), has been found to possess a number of valuable features for general use and has in the course of several years displaced other mixers in this laboratory. It is briefly described to make constructional details generally available.

Figure 1 shows a photograph of the G.R.L. mixer with an open and a closed bowl beside it. Both bowls are made of tin-plated spun brass and are jacketed for water circulation. The open bowl is used for routine mixing of dough in air. It is positioned into a split ring forming a ring clamp which is tightened by means of the lever seen in front of the mixer. The three large openings or windows are provided for free circulation of air and so that dough may be observed during mixing.

The closed bowl is used for mixing dough in an inert atmosphere. To obtain an air-tight seal, vacuum wax is applied to the flat surface of the rim, the bowl is pushed up snugly against a rubber gasket, and the clamp is tightened. A completely closed mixing chamber is thus obtained. The bowl is provided with an inlet tube through which the doughing liquid is added. Two additional connections with the mixing chamber are provided above the bowl through the body of the mixer. One of these can be seen on the right-hand side in the photograph. Normally, one of these outlets is connected to a vacuum pump

[1] Manuscript received September 30, 1954. Paper No. 138 of the Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Manitoba, and No. 315 of the Associate Committee on Grain Research (Canada).

and the other to a tank of nitrogen. In one mixer, designed for use at pressure higher than atmospheric, the bowl was further secured to the body of the mixer by swivel screw clamps.

The mixing action is provided by one curved and two straight pins. This action may best be explained by considering the gear system which drives the mixing pins. Figure 2 shows the mechanism viewed from below. The following main parts may be readily identified in the photograph: the drive plate (A) above the internal spur

Fig. 1. G.R.L. dough mixer with an open and a closed bowl.

gear (B) around the periphery; two large spur gears (C 1) and (C 2) mounted concentrically above one another; and a group of three small spur gears (D). The drive plate is connected to a vertical drive shaft of a 1 to 30 worm gear reducer. As the drive plate turns, the upper large gear (C 1), which carries the curved pin, travels clockwise around the periphery on the internal gear (B) and at the same time rotates about its own axis. Through a reducer-idler combination of three

small gears (D) the upper large gear drives a lower large gear (C 2) which carries the two straight pins. Since the two large gears have a common center, their travel around the mixer is identical, but they rotate in opposite directions and at different speeds.

Dough is mixed between the pins as they pass one another. The curved pin adopted from Malloch's mixer (4) effectively serves to prevent the dough from climbing up on the pins even during prolonged

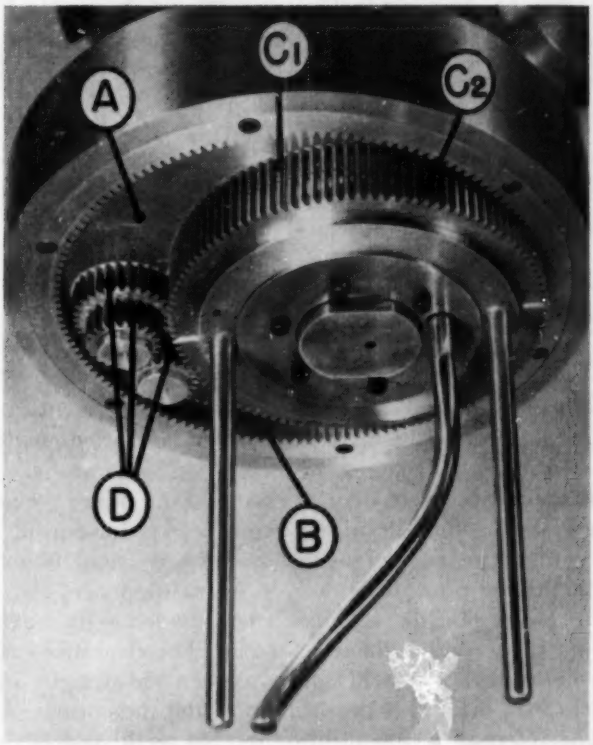

Fig. 2. Gear mechanism of the mixer viewed from below.

mixing. The pattern traced by the mixing pins is shown in Fig. 3. The straight pin describes, alternately a large loop, then a small loop. The two loops are completed in two revolutions of the drive plate and the pin advances by the distance shown between the solid and dotted small circles. Both straight pins trace identical paths one cycle apart. The curved pin traces a four-looped path around the bowl. In two turns of the drive plate, however, only the distance shown between the solid and dotted small circles is traversed by the pin. The entire

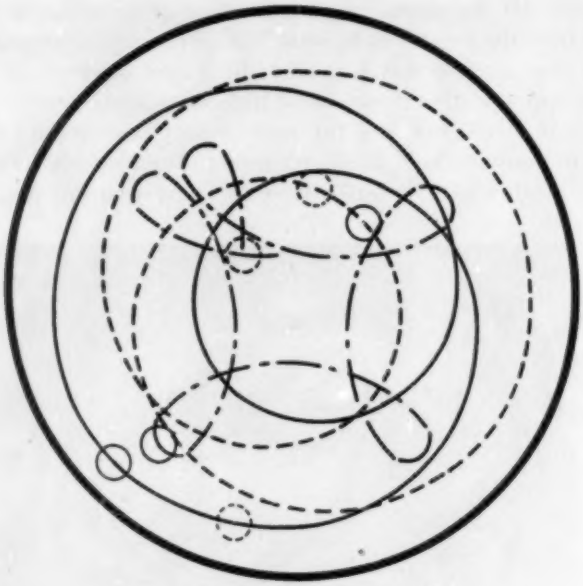

Fig. 3. Pattern traced by the mixing pins.

pattern advances around the bowl. It returns to an identical position only after 630 turns of the drive plate which is approximately after 10 minutes of mixing.

Essential dimensions of the main parts of the mixer are as follows: The bowl measures 5½ in. inside diameter, 6½ in. outside diameter and is 4¾ in. deep. It is designed to mix doughs from 100 to 200 g. of flour. The mixing pins, made of 5/16 in. stainless steel rod, are 5 in. long. The curved pin is a section of a left handed helix making a half turn in 4¼ in. around a diameter of 2 in. The clearance between the straight pins and the bowl is ¼ in., between the straight and curved pins 1/8 to 3/16 in., and between the pins and the bottom of the bowl ¼ in. The internal gear is bronze, has 144 teeth, and is 6 in. pitch diameter. The two large gears, the upper of which is steel and the lower is bronze, have 110 teeth and a pitch diameter of 4.583 in. The three small gears are steel. The two reduction gears have 30 and 21 teeth and pitch diameters of 1.250 and .875 in. The idler has 20 teeth and .833 pitch diameter, but its dimensions are of no direct interest. All gears have ⅜ in. face.

The G.R.L. mixer is mounted on a stand of welded aluminum plate and is enclosed in sheet aluminum housing. It is powered by a ¼ h.p. 1725 r.p.m. motor by means of a V-belt drive and variable

pitch pulleys. The speed adopted is 36 r.p.m. at the drive plate of the mixer. Other speeds may be obtained by adjusting the pulleys.

The mixing action of the G.R.L. mixer is slightly more vigorous than that of the Hobart with two hooks. After each mix the bowl of the G.R.L mixer remains remarkably clean and is ready for the next mix. The dough is mixed continuously and does not disengage itself from the mixing pins for indefinite periods as in the Hobart mixer. The action is much gentler than that of the Swanson mixer. Baking trials have shown that 3 minutes on the Hobart, 2.75 minutes on the G.R.L., and 1 minute on the Swanson mixer, give approximately comparable amounts of mixing.

Acknowledgments

The authors are indebted to Messrs. H. E. Rasmussen and V. Martens who designed the mixer, and to Messrs. H. E. Hughes and K. J. Rerie who have made the recent models.

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COMMUNICATION TO THE EDITOR

An Improved Wheat Prediction Test for Macaroni Quality

DEAR SIR:

A method of predicting the pigment content of macaroni from measurements of the lipoxidase activity and pigment content of ground whole wheat has been developed in this laboratory (Cereal Chem. **30:** 334–342. 1953). Further experience since publication of this method, covering a large number of samples, has shown that for some wheats lipoxidase activity is so high that the substrate, in the lipoxidase assay as outlined for wheat, becomes the limiting factor. As a result of this finding, the amount of enzyme extract used for the test has been cut in half (to 0.4 ml., previously 0.8 ml.), and the determination is made in 50-ml. Warburg flasks in place of 15-ml. flasks. This latter change is merely a matter of convenience and is not a mandatory change in the method.

Data on lipoxidase activity, using this modification, and on pigment contents of wheat and of the macaroni processed from it, have been obtained for 137 wheat samples. The multiple regression relating macaroni pigment to both wheat lipoxidase and wheat pigment has been recalculated from these data and a revised prediction equation has been obtained:

Correlations

Wheat pigment vs. macaroni pigment: $r = 0.89$; $s = 0.67$

Wheat lipoxidase vs. macaroni pigment: $r = -0.72$

Wheat lipoxidase vs. wheat pigment: $r = -0.51$

Regression equation

$$P_m = 0.807\ P_w - 0.0105\ L_w - 0.383 \qquad s = 0.48,$$

where P_m is macaroni pigment, p.p.m.,

P_w is wheat pigment, p.p.m., and

L_w is lipoxidase activity of wheat in $\mu 10_2$/min/g.

A wider variety of wheat samples was available for this study than for the previous one, and the multiple correlation coefficient for the new equation is 0.944 as compared with 0.910 for the previous equation. Although the standard error of prediction (s) with the new equation is identical to that obtained previously (0.48 p.p.m.), the new equation should give more satisfactory results over a wide range of wheat lipoxidase values.

November 9, 1954

G. N. IRVINE
J. A. ANDERSON
Grain Research Laboratory
Board of Grain Commissioners for
Canada, Winnipeg, Manitoba

BOOK REVIEW

Business without Boundary: The Story of General Mills, by James Gray. xiii+320 pp.; indexed. University of Minnesota Press, Minneapolis, Minn. Price, $4.75.

As a rule, business histories lack the fast movement and humor of other non-fiction pieces written to provide lively entertainment. However, this reviewer is happy to report that James Gray's *Business without Boundary* is a notable exception.

In telling the story of General Mills Mr. Gray makes full use of his background as literary critic, novelist, and popular historian. The account begins with the founding of the Washburn "B" mill in 1866 by Cadwallader Washburn and traces the growth of that company through the Washburn-Crosby merger in 1879 to the organization of General Mills in 1928. The names of John Crosby, Cadwallader Washburn, William Hood Dunwoody, and James Stroud Bell occupy a prominent position in the earlier sections of Gray's book, while those of James Ford Bell, Harry A. Bullis, C. H. Bailey, Samuel Chester Gale, and Marjorie Child Husted, to name but a few, are associated with the organization and growth of General Mills.

Mr. Gray makes it clear that *Business without Boundary* is not just the story of industrial growth in America; it is also the story of industrial maturity—of great leadership, during periods of depression as well as prosperity. This leadership was exemplified by General Mills' first head and founder, James Ford Bell. Mr. Bell's gift of timing, his sense of humor coupled with his patience and foresight, enabled GMI to brave many an economic storm which would have downed a lesser company.

While only 26 years old, General Mills has an enviable list of "firsts," Gray points out, both in the technical field and in the area of sales and merchandising. In addition to GMI's pioneering work on vitamins, biscuit mixes, breakfast cereals, etc., they pioneered the use of radio as a sales medium; they were the first (and perhaps a dubious honor) to introduce the singing commercial; among the first in the milling field to make large use of the premium; the first to hand-tailor soap opera for children. Ad men at GMI learned early the value of winning over the child as an indirect but successful approach to the parents. In their first effort GMI sponsored a radio version of the comic strip "Skippy" and later switched to "Jack Armstrong," the creation of Robert Andrews, script writer for the Skippy show. The success of the "All-American Boy" and the "Breakfast of Champions" hardly needs to be retold. Likewise, GMI's advertising symbol, Betty Crocker, has reached the pinnacle of public recognition by being voted the second-best-known "woman" in America.

Business without Boundary is an interesting, fast-moving story of a great company in a colorful business. The story of General Mills is also the story of American milling, with special emphasis on the Minneapolis area. Gray's book should find a place on every cereal chemist's bookshelf as a volume to read and enjoy.

R. J. Tarleton
Managing Editor, *Cereal Chemistry*
University Farm
St. Paul, Minnesota

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

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SUGGESTIONS TO AUTHORS

General. Authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6:1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style,* University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10°C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

For more detailed information on manuscript preparation see *Cereal Chem.* **30:** 351–352 (1953).

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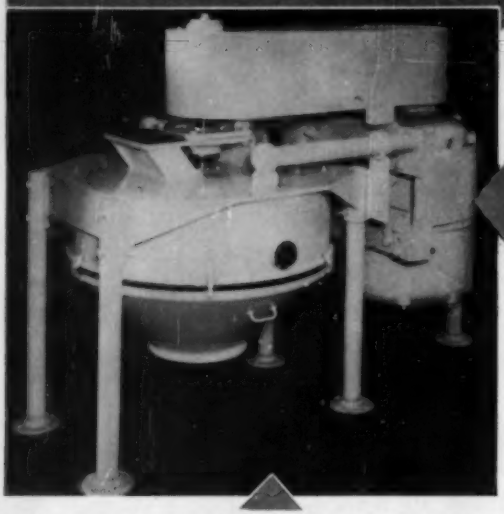

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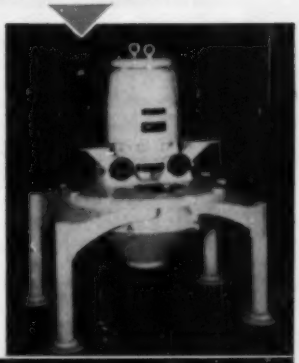

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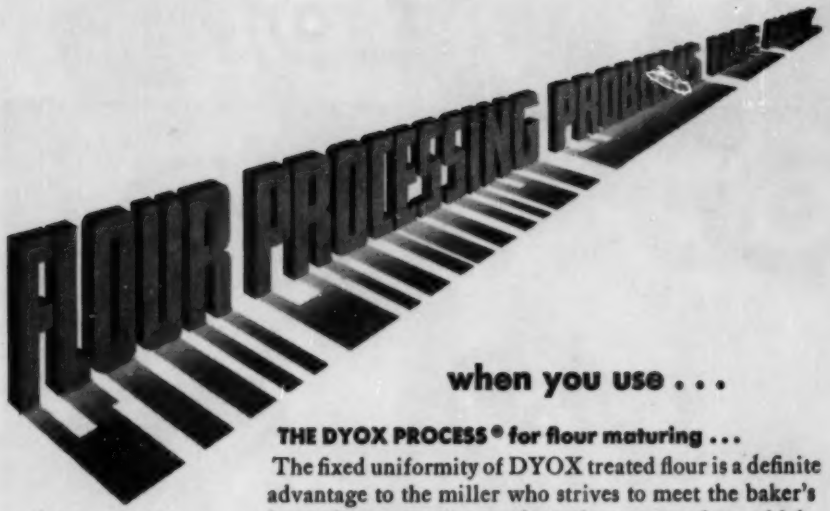

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